Meeting Title:	**Subcommittee on Antimicrobial Susceptibility Testing (AST)**	**Contact:**	egomez@clsi.org
Meeting Location:	Orlando, Florida, USA		
Meeting Dates and Times: All times are Eastern Standard (US) time.	**Plenary 1:** Monday, 27 January 2025, 7:30 AM – 12:00 PM **Plenary 2:** Monday, 27 January 2025, 1:00 – 5:30 PM **Plenary 3:** Tuesday, 28 January 2025, 7:30 AM – 12:00 PM		
Meeting Purpose:	The purpose of this meeting is to review and discuss AST WG and SC business in preparation for publication of the next edition of CLSI M100 (36th).		
Requested Attendee(s):	SC Chairholder, Vice-Chairholder, Secretary, Members, Advisors, and Reviewers; Expert Panel on Microbiology Chairholder and Vice-Chairholder; Other Interested Parties; CLSI Staff		

Attendee(s):	
Amy J. Mathers, MD, D(ABMM) **AST Subcommittee Chairholder**	**University of Virginia Medical Center**
James S. Lewis, II, PharmD, FIDSA **AST** Subcommittee Vice-Chairholder	**Oregon Health and Science University**
Alexandra L. Bryson, PhD, D(ABMM) AST Subcommittee Secretary	**Virginia Commonwealth University Health**
Members Present:	
Kevin Alby, PhD, D(ABMM)	University of North Carolina Hospital
April M. Bobenchik, PhD, D(ABMM)	Penn State Health, Milton S. Hershey Medical Center
Shelley Campeau, PhD, D(ABMM)	Scientific and Medical Affairs Consulting, LLC
Tanis Dingle, PhD, D(ABMM), FCCM	Alberta Precision Laboratories
German Esparza, MSc	Proasecal SAS Columbia
Mark Fisher, PhD, D(ABMM)	ARUP
Stephanie Mitchell, PhD, D(ABMM)	Cepheid
Navaneeth Narayanan, PharmD, MPH	Rutgers University, Ernest Mario School of Pharmacy
Elizabeth Palavecino, MD	Wake Forest University School of Medicine
Virginia M. Pierce, MD, FIDSA	University of Michigan Medical School
Audrey N. Schuetz, MD, MPH, D(ABMM)	Mayo Clinic (Rochester, MN)
Patricia J. Simner, PhD, D(ABMM)	Johns Hopkins University School of Medicine, Department of Pathology
Pranita D. Tamma, MD, MHS	John Hopkins University School of Medicine, Department of Pediatrics
Members Absent:	
Joseph D. Lutgring, MD	Centers for Disease Control and Prevention
Advisors Present:	
Mariana Castanheira, PhD	Element/JMI Laboratories
Sharon K. Cullen, BS, RAC	Beckman Coulter, Inc. Microbiology Business
Lindsay Donohue, PharmD, BCIDP	University of Virginia Medical Center
Rebekah Dumm, PhD, D(ABMM)	Washington University School of Medicine
Andrea L. Ferrell, MLS(ASCP) ^{CM}	BD
Sören Gatermann, PhD	EUCAST
Elizabeth Hirsch, PharmD	University of Minnesota
Andre Hsiung, M(ASCP), MS, MBA	Hardy Diagnostics
Romney M. Humphries, PhD, D(ABMM), FIDSA, FAAM	Vanderbilt University Medical Center
Antonieta Jimenez Pearson, MQC, PhD	INCIENSA

Kristie Johnson, PhD, D(ABMM)	University of Maryland
Thomas J. Kirn, Jr., MD, PhD	Rutgers Robert Wood Johnson Medical School
William Miller, MD	Methodist Hospital
Samia Naccache, PhD, M(ASCP)^{CM}, D(ABMM)	LabCorp
Mike Satlin, MD	Weill Cornell Medicine
Jolyn Tenllado	bioMérieux
Melvin P. Weinstein, MD	Robert Wood Johnson University Hospital
Katsunori Yanagihara, MD, PhD	Japanese Society for Clinical Microbiology
Advisors Absent:	
Amelia S. Bhatnagar, MPH	Centers for Disease Control and Prevention
Marcelo Galas, BSc	Pan American Health Organization
Holly Huse, PhD, D(ABMM)	Harbor UCLA Medical Center
Dmitri Iarikov, MD, PhD	FDA Center for Drug Evaluation and Research
Joe Kuti, PharmD, FIDP, FCCP	Consultant
Maria Machado	Centers for Disease Control and Prevention
Brigit Quinn, MS	SeLux
Ribhi Shawar, PhD, D(ABMM), F(AAM)	FDA Center for Devices and Radiological Health
Barbara L. Zimmer, PhD	Beckman Coulter
Reviewers and Guests (Non-SC-roster attendees): see Plenary Attendee List below	
Staff:	
Jennifer Adams, MT(ASCP), MSHA	CLSI
Emily Gomez, MS, MLS(ASCP) ^{CM} MB ^{CM}	CLSI
Barb Jones, PhD	CLSI
Christine Lam, MT(ASCP)	CLSI
Lori Selden, MS, MT(ASCP)	CLSI

Plenary Agendas

PLENARY AGENDA: SESSION 1 – IN PERSON Monday, 27 January 2025 7:30 AM - 12:00 PM Eastern Standard Time (US)			
Time	**Item**	**Presenter**	
7:30 AM – 7:40 AM (10 min)	Opening Remarks	A. Mathers	6
7:40 AM – 7:45 AM (5 min)	Approval of Meeting Agenda	A. Mathers	6
7:45 AM – 7:55 AM (10 min)	CLSI Welcome and Update	J. Adams	6
7:55 AM – 8:05 AM (10 min)	CLSI Awards	B. Jones	7
8:05 AM – 8:15 AM (10 min)	AST Subcommittee Update	E. Gomez	8
8:15 AM – 8:25 AM (10 min)	Antifungal AST Subcommittee Update	P. Dufresne N. Wiederhold	13
8:25 AM – 8:35 AM (10 min)	EUCAST Update	S. Gatermann	16
8:35 AM – 9:05 AM (30 min)	Joint CLSI-EUCAST WG	J. Hindler E. Matuschek	18
9:05 AM – 9:35 AM (30 min)	Anaerobe WG	D. Carpenter S. Copsey-Mawer	25
9:35 AM – 9:55 AM (20 min)	Break		
9:55 AM – 11:25 AM (1 hr 30 min)	Quality Control WG	S. Cullen C. Pillar	29
11:25 AM – 12:00 PM (30 min)	Table 1 Placement	S. McLeod	43

PLENARY AGENDA: SESSION 2 – IN PERSON Monday, 27 January 2025 1:00 PM - 5:30 PM Eastern Standard Time (US)			
Time	**Item**	**Presenter**	

1:00 PM – 3:00 PM (2 hr)	Breakpoints WG: Part 1	N. Narayanan M. Satlin	50
3:00 PM – 3:20 PM (20 min)	Break		
3:20 PM – 5:30 PM (2 hr)	Breakpoints WG: Part 2	N. Narayanan M. Satlin	
PLENARY AGENDA: SESSION 3 – IN PERSON Tuesday, 28 January 2025 7:30 AM - 12:00 PM Eastern Standard Time (US)			
Time	**Item**	**Presenter**	
7:30 AM – 9:30 AM (2 hr)	Methods WG: Part 1	T. Dingle K. Johnson	85
9:30 AM – 9:50 AM (20 min)	Break		
9:50 AM – 10:50 AM (1 hr)	Methods WG: Part 2	T. Dingle K. Johnson	
10:50 AM – 11:20 AM (30 min)	Text and Tables WG	A. Bobenchik S. Campeau	115
11:20 AM – 11:50 AM (30 min)	Outreach WG	J. Hindler A. Schuetz	120
11:50 AM – 12:00 PM (10 min)	M45 WG	R. Humphries T. Simner	125
12:00 PM	Closing Remarks	A. Mathers	128

Summary of Voting Decisions and Action Items

Summary of Passing Votes			
#	**Motion Made and Seconded**	**Results**[a]	**Page**[b]
1.	To approve the January 2025 meeting agenda.	13-0-0-1	6
2.	To approve the Forward revisions in CLSI M23S3 as proposed.	13-0-0-1	19
3.	To approve the reaffirmation of CLSI M11.	12-0-0-2	25
4.	To endorse the review of previously published data and analyze the published data using CLSI M23 methods requirements for disk diffusion QC for anaerobes.	13-0-0-1	28
5.	To accept the contezolid MIC QC ranges for *Staphylococcus aureus* ATCC 29213 (1-4 µg/mL), *Enterococcus faecalis* ATCC 29212 (0.5-4 µg/mL), and *Streptococcus pneumoniae* ATCC 49619 (0.5-2 µg/mL).	13-0-0-1	30
6.	To accept the contezolid disk (5 µg) diffusion QC ranges for *Staphylococcus aureus* ATCC 25923 (17-23 mm) and *Streptococcus pneumoniae* ATCC 49619 (17-23 mm).	13-0-0-1	31
7.	To accept the zosurabalpin disk (5 µg) diffusion QC range for *Acinetobacter baumannii* NCTC 13304 (22-28 mm) with a footnote and picture instructing to read the outer zone diameter.	13-0-0-1	33
8.	To archive the Tier 3 inquiry for the *Streptococcus pneumoniae* ATCC 49619 ceftriaxone MIC QC range (0.03-0.12 µg/mL).	13-0-0-1	36
9.	To accept the imipenem MIC QC range for *Klebsiella pneumoniae* ATCC BAA-1705 (4–32 µg/mL).	13-0-0-1	38
10.	To accept the imipenem MIC QC range for *Klebsiella pneumoniae* ATCC BAA-2814 (≥16 µg/mL).	13-0-0-1	38
11.	To move sulbactam-durlobactam to Tier 3 cascading off carbapenems in Table 1B-2 (*Acinetobacter* spp.).	13-0-0-1	47
12.	To remove the doxycycline breakpoints for *Acinetobacter* spp.	12-1-0-1	59
13.	To accept the proposed minocycline comment for *Acinetobacter baumannii* with wordsmithing from Text and Tables Working Group.	13-0-0-1	61
14.	To accept the disk (30 µg) diffusion cefiderocol susceptible breakpoint (≥17 mm) for *Stenotrophomonas maltophilia*.	13-0-0-1	73
15.	To form an ad hoc working group under the Joint CLSI EUCAST Working Group to provide guidance on deviations from the reference method.	12-0-0-2	105
16.	To accept the proposed edits and comments to Table H1.2 with modified language in step 10 to change “cation” to “iron”.	12-0-0-2	111
17.	To remove the “see comment 21” in the comments column for cefuroxime, loracarbef, cefaclor, cefdinir, cefpodoxime, and cefprozil in Table 2A-1.	12-0-0-2	118

[a] Key for voting: X-X-X-X = For-against-abstention-absent
[b] Page links can be used to go directly to the related topic presentation and voting discussions.

2025 JANUARY AST MEETING SUMMARY MINUTES PLENARY 1: Monday, 27 January 7:30 AM – 12:00 PM Eastern Standard Time (US)	
#	**Description**
1.	**OPENING REMARKS (A. MATHERS)** Dr. Mathers opened the meeting at 7:30 AM Eastern Standard (US) time by welcoming the participants to the hybrid CLSI meeting in Orlando, Florida.
2.	**APPROVAL OF MEETING AGENDA**
	A motion to approve the January 2025 meeting agenda was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)
3.	**CLSI WELCOME AND UPDATE (B. JONES)** Dr. Jones thanked the CLSI experts for their ongoing support, time, and efforts.

4.	**CLSI AWARDS (B. JONES)** Dr. Jones presented CLSI awards to: • John V Bergen Excellence Award: Susan Sharp, PhD, D(ABMM), F(AAM) • Excellence in Standards Development Award: Ribhi M. Shawar, PhD, D(ABMM), and F(AAM)

5. **AST SUBCOMMITTEE UPDATE (E. GOMEZ)**

Ms. Gomez provided an update on the AST Subcommittee. The main points included:

- 2025 Roster Changes
  - AST Subcommittee Chairholders and Secretary
    - Chairholder: Amy J. Mathers, MD, D(ABMM)
    - Vice-Chairholder: James S. Lewis II, PharmD, FIDSA
    - Secretary: Alexandra L. Bryson, PhD, D(ABMM)
  - AST Subcommittee Members
    - Rotating Off
      - Sharon Cullen
      - Romney Humphries
      - Thomas Kirn
      - Susan Sharp
      - Melvin Weinstein
    - New Members
      - Kevin Alby
      - April Bobenchik
      - Shelley Campeau
      - Mark Fisher
      - Stephanie Mitchell
  - AST Subcommittee Advisors
    - Rotating Off
      - Tanaya Bhowmick
      - Christian Giske
      - Howard Gold
      - Natasha Griffin
      - Janet Hindler
      - Linda Miller
      - Greg Moeck
      - Kiyofumi Ohkusu
      - Eric Wenzler
    - New Advisors
      - Sharon Cullen
      - Rebekah Dumm
      - Sören Gatermann
      - Elizabeth Hirsch
      - Andre Hsiung
      - Romney Humphries

- Holly Huse
- Kristie Johnson
- Thomas Kirn
- William Miller
- Samia Naccache
- Brigit Quinn
- Ribhi Shawar
- Jolyn Tenllado
- Katsunori Yanagihara
- Melvin Weinstein

- Working Group Changes
  - Methods Working Group
    - Chairholders: Tanis Dingle and Kristie Johnson
    - Combined the Methods Application and Interpretation Working Group and the Methods Development and Standardization Working Group
    - Thank you to Thomas Kirn and Barbara Zimmer!
  - Anaerobe Working Group
    - Chairholders: Darcie Carpenter and Sarah Copsey-Mawer
    - Formerly an ad hoc working group under Methods Application and Interpretation Working Group
- Ad Hoc Working Group (AHWG) Changes
  - Disbanded Ad Hoc Working Groups
    - Methods Working Group
      - Disk Diffusion Reference vs Standard Method AHWG
      - AST of Non-Enterobacteriaceae AHWG
  - New Ad Hoc Working Groups
    - Breakpoints Working Group
      - Aztreonam-Avibactam AHWG
      - Trimethoprim-Sulfamethoxazole Beta-Hemolytic Streptococci AHWG
    - Methods Working Group
      - Appendix A AHWG
      - Early Growth AST AHWG
      - Intrinsic Resistance Definition AHWG
- Subcommittee Voting Rules
  - 2/3 majority of members to approve
  - 1 vote to approve from each constituency (professions, industry, government)

Subcommittee on Antimicrobial Susceptibility Testing
Chairholder's Rules on Voting

January 2025 AST Subcommittee Roster
14 voting members (excludes Chairholder and Vice-chairholder)

Committee Status	"Pass" Vote
All members present and voting	14-0; 13-1; 12-2; 11-3; 10-4; 9-5
One member not present or abstaining	13-0; 12-1; 11-2; 10-3; 9-4
Two members not present or abstaining	12-0; 11-1; 10-2; 9-3
Three members not present or abstaining	11-0; 10-1; 9-2
If more than three members not present	Chairholder's discretion to conduct vote or table until sufficient members are present, or an electronic vote is taken.

Guidance on Considerations of Conflicts of Interest by Subcommittee Members Voting on an Issue

On any subcommittee business for which a subcommittee vote is required, all subcommittee members are expected to cast a vote, from the following voting options:

- Accept
- Accept with comments, and/or qualifications
- Reject with specified supporting reason(s)
- Abstain due to conflict of interests*

*Any personal gain within 3 years or imminently expected as a result of working with a specific drug (occasionally might apply if did such work with direct competitor[s]).

Note: "Personal gains" do not include payments only to your institution or research funds. These need to be declared but do not require a declared abstention.

- AST Subcommittee Documents
  - 2024 Publications
    - M02, Performance Standards for Antimicrobial Disk Susceptibility Tests, 14th Edition
    - M07, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 12th Edition

- M23S, Procedure for Optimizing Disk Contents (Potencies) for Disk Diffusion Testing of Antimicrobial Agents Using Harmonized CLSI and EUCAST Criteria, 2nd Edition
- M23S2, Process to Submit Disk Content (Potency) Data for Joint CLSI-EUCAST Working Group Review and Approval, 2nd Edition

  - 2025 Publications
    - M100-35th Edition published in January 2025
  - Active Documents
    - M45, Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria, 4th Edition
    - Chairholders: Trish Simner and Romney Humphries
  - Call for Volunteers
    - M24, Susceptibility Testing of Mycobacteria, *Nocardia* spp. and Other Aerobic Actinomycetes, 4th Edition
    - Chairholders: Nancy Wengenack and Nikki Parrish
    - Apply by 5 February 2025

- Next Meeting
  - 31 May – 3 June 2025 in Dallas, Texas
  - Meeting materials due 2 May 2025
  - Virtual Only Working Group Meetings in weeks of 12 May and 19 May 2025
- CLSI M100 36th Edition
  - Publishing January 2026
  - January and June 2025 meeting decisions
  - No additional decisions after the June 2025 meeting
- AST Subcommittee Mission Statement (Presented by Dr. Mathers)

• Develop standard reference methods for antimicrobial susceptibility tests.

• Provide QC parameters for standard test methods.

• Establish breakpoints and interpretive categories for the results of standard antimicrobial susceptibility tests and provide epidemiological cutoff values when breakpoints are not available.

• Provide suggestions for testing and reporting strategies that are clinically relevant and cost-effective.

• Continually refine standards and optimize detection of emerging resistance mechanisms through development of new or revised methods, breakpoints, and QC parameters.

• Educate users through multimedia communication of standards and guidelines.

• Foster a dialogue with users of these methods and those who apply them.

The ultimate purpose of the subcommittee's mission is to provide useful information to enable laboratories to assist the clinician in the selection of appropriate antimicrobial therapy for patient care. The standards and guidelines are meant to be comprehensive and to include all antimicrobial agents for which the data meet established CLSI guidelines. The values that guide this mission are quality, accuracy, fairness, timeliness, teamwork, consensus, and trust.

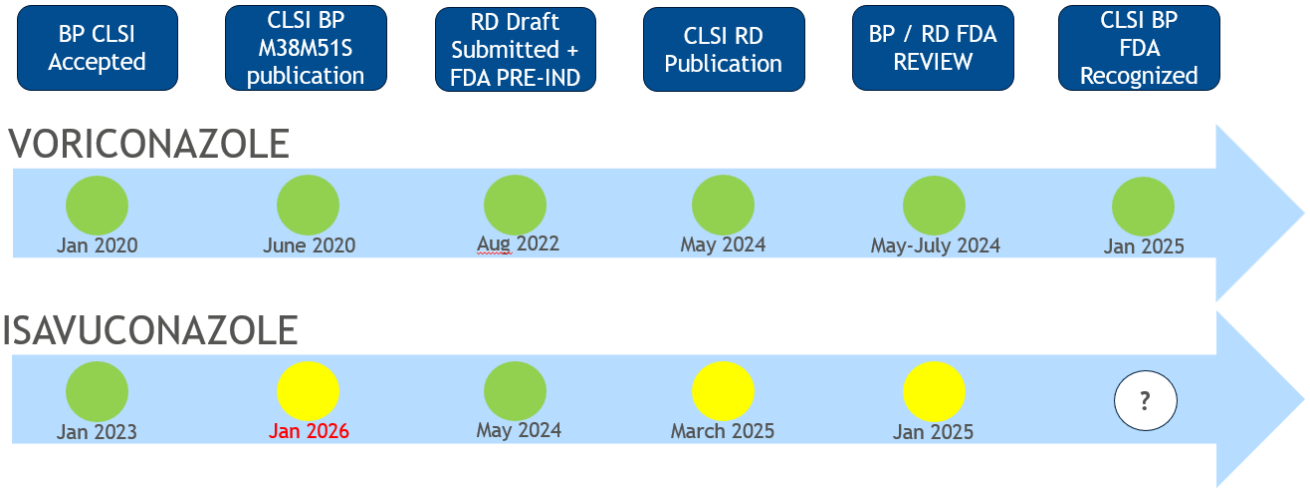
- CLSI Tribute to Karla Tomfohrde (Presented by Dr. Cullen)

	○ 1946 - 2024 ○ Started career at CDC focusing on bacterial identification ○ Recruited into industry (API, MicroScan) ○ Long time CLSI contributor ▪ CLSI AST Advisor representing AST industry (early 1990's) ▪ First CLSI QCWG Chair ▪ Coordinated 1991 QC Pilot study to improve CLSI M23 Tier 2 study design based on statistical confidence, risks, costs, and practical logistics (7 laboratories, 10 replicates, 3 media lot, 2 disk lots). ○ Conducted numerous global educational sessions on ID/AST and updated CLSI guidelines. ○ Worked with Dr Clyde Thornsberry at MRL (later Eurofins). ▪ Set up and maintained early global AST surveillance program. ○ Proud Florida State alumni. ○ Loved watching sports, needlepoint and traveling (went to all 7 continents) ○ We continue to benefit from all her contributions.

6. **ANTIFUNGAL AST SUBCOMMITTEE UPDATE (P. DUFRESNE)**

Dr. Dufresne provided an update on the activities of the Antifungal AST Subcommittee (AFSC). The main points included:

- Antifungal Subcommittee Working Groups
  - Reporting Working Group
  - Breakpoint Working Group
  - ECV Working Group
  - Document Review Working Group
  - MIC Reading Working Group – New
- Reporting Working Group
  - Intrinsic resistance
  - Body site reporting
  - New Intrinsic Resistance Definition AHWG
    - Combined effort of our 3 CLSI susceptibility testing groups
    - AFSC also working on a reduced antifungal susceptibility definition
  - New potential project: what antifungals to test and report (like CLSI M100 Table 1)
- Breakpoint Working Group
  - *Aspergillus fumigatus* azoles breakpoints



    - Posaconazole
      - Breakpoint not yet established (met with Merck in November 2022)
      - Concerns regarding interlaboratory MIC variability (multi-laboratory study to start soon)
    - First mold FDA approved breakpoint – Voriconazole for *Aspergillus fumigatus*
  - Rezafungin Breakpoints
    - Long half-life echinocandin (FDA approved)
    - CLSI susceptible breakpoints reaffirmed in 2024 (distinct from FDA's breakpoint)

- 7 Candida species (including *Candida auris*)
- Rationale document draft completed – under AFSC review (Jan 2025)

- ECV Working Group
  - New ECVs submitted
    - *Sporothrix* spp. (subcutaneous/ zoonotic)
      - 8 ECVs
      - 3 species (*S. schenckii, S. brasiliensis, S. globosa*)
    - *Fonsecaea pedrosoi* (No. 1 chromoblastomycosis agent)
      - 6 ECVs – Manuscript accepted in JCM
      - Data being gathered for *F. monophora* and *Phialophora*
    - + 29 ECVs waiting to be published in CLSI M57S
      - Rare *Candida*, *Scedosporium*, *Lomentospora*, …)
- Document Review Working Group
  - 6 Antifungal Methods and Supplements Under Review
    - CLSI M27 and CLSI M38
      - Complete 5 year review
      - Launch: March-May 2024
      - Publication: April-May 2026
    - CLSI M44
      - Fast track limited revision
      - Launch: March 2024
      - Publication: May 2026
    - CLSI M27M44S, CLSI M38M51S, and CLSI M57S
      - Edaptive review started September 2024-January 2025
      - Expected publication: January- May 2026
- MIC Reading Working Group
  - Provide guidance
    - Define how to read (MIC, MEC, zone diameter)
    - How to read in difficult MICs situation
      - skip well, paradoxical growth, trailing, heteroresistance, etc
  - In the antifungal documents
    - Better defined in text
    - Provide good example photos (text body, appendices)
  - Create quick guides (like CLSI M02/M07 Quick Guides of the AST Subcommittee)
  - Provide training in interactive web interface – free course
    - CLSI training on board
- Novel Antifungals
  - SCY-247 from Scynexis
    - Second generation triterpernoid (Ibrexafungerp)

	▪ Broad antifungal activity (yeast, mold and dimorphics) ○ Opelconazole from Pulmocide (phase III trial) ▪ Target: *Aspergillus* spp. ▪ Administration: Oral inhalation (nebulization) ▪ Tier 1 - QC ranges submitted to Subcommittee **SC DISCUSSION (MAIN POINTS)** • Is the Antifungal Subcommittee working on *Trichophyton indotineae* AST? ○ Not currently. There is more data becoming available, so something to be considered in the future.

7. **EUCAST UPDATE (S. GATERMANN)**

Dr. Gatermann provided an update on the activities of EUCAST. The main points included:

- 2025 EUCAST Subcommittee
  - Christian G. Giske, chair until May 1st, 2024, Sören G. Gatermann, since May 2nd, 2024
  - Mandy Wootton, scientific secretary
  - Rafael Cantón, clinical data coordinator
  - Gunnar Kahlmeter, technical data coordinator/webmaster
  - Shampa Das, PK-PD expert
  - Joseph Meletiadis, PK-PD expert
  - Christoffer Lindemann, Norway
  - Alasdair MacGowan, UK
  - Marlène Amara, France
  - Erika Matuschek, Sweden, head of the EDL
  - Barbara Holzknecht, Denmark (May 2023- Apr 2025)
  - Anouk Muller, The Netherlands (May 2023- Apr 2025)
- Consultations and new breakpoints during 2024
  - Criteria for evaluation and reporting in three non-fermentative species for cefiderocol
    - No formal classification as S-I-R
  - New Guidance for fluoroquinolones, tetracyclines, ceftazidime, and cefepime for *Stenotrophomonas maltophilia*
  - Penicillins vs *Streptococcus pneumoniae* update in V 14.0 (2024)
  - Viridans streptococci breakpoints for endocarditis
  - Enterococci
    - breakpoints are valid for almost all enterococci
    - needed some minor adaptations in the tables
  - New antibiotics

Antibiotic	Species	S ≤	R >
Aztreonam-Avibactam	Enterobacterales	4	4
	Pseudomonas	IE	IE
	Stenotrophomonas	IE	IE
Cefepime-Enmetazobactam	Enterobacterales	4	4
	Pseudomonas	Note	Note

Note: the addition of the inhibitor does not add clinical benefit

  - Joint CLSI-EUCAST Working Group is working on harmonization at twice yearly meetings
- Upcoming consultations 2025
  - trimethoprim-sulfamethoxazole
  - EUCAST dosing tab adapted to pediatric use

	SC DISCUSSION (MAIN POINTS)
	• Laboratories do not know if patients have endocarditis or not. How do laboratories communicate the two different breakpoints?
	  ○ Call the physician to ask or report both breakpoints.
	• How did EUCAST come up with the endocarditis breakpoints? Did you have PK/PD data?
	  ○ Many decisions were not PK/PD based as there was no new data; the decisions were based on clinical studies. EUCAST has an endocarditis rationale document available on their website.

8. **JOINT CLSI EUCAST WORKING GROUP (J. HINDLER AND E. MATUSCHEK)**

JOINT WORKING GROUP GOALS

- Goal #1: Describe a method for disk content determination which can be used early in the drug development process to avoid having different disk contents in the CLSI and EUCAST standards.
- Goal #2: Discuss differences between CLSI and EUCAST QC criteria, methods for establishing QC criteria and the possibility of harmonizing CLSI and EUCAST QC criteria.
- Expand goals beyond disk content and QC criteria?

TOPICS MOVED TO OTHER WORKING GROUPS

- Topics originating in Joint Working Group which will be discussed at other working groups during the CLSI AST Subcommittee Winter 2025 meetings:
  - QC ranges for ß-lactam agents vs ß-lactam/ß-lactamase inhibitor combinations for susceptible QC strains – QC Working Group
  - Colony counts – Methods Working Group -> Will move forward with in the Joint Working Group
  - Modification of reference BMD to accommodate agents that perform poorly in routine CAMHB – Methods Working Group

JOINT WORKING GROUP DOCUMENTS

- M23S 2nd Edition (published November 2024)
  - Procedure for Optimizing Disk Contents (Potencies) for Disk Diffusion Testing of Antimicrobial Agents Using Harmonized CLSI and EUCAST Criteria
- M23S2 2nd Edition (published November 2024)
  - Process to Submit Disk Content (Potency) Data for Joint CLSI-EUCAST Working Group Review and Approval
- M23S3 1st Edition (published June 2023)
  - Procedure for Confirming the Acceptability of Mueller-Hinton Agar Sources for Subsequent Use in CLSI and/or EUCAST Studies to Establish Disk Diffusion Quality Control Ranges
- Available on the CLSI AST Micro Free website and on the EUCAST website as SOPS 11, 12, and 13.

ADDITION TO CLSI M23S3

- Background:
  - The Joint Working Group discussed the possibility of adding recommendations for preQC of CAMHB to M23S3 1st Edition. The recommendations would be similar to those for preQC of MHA.
  - After much discussion it was decided that rather than add detailed instructions to M23S3 1st Edition, Joint Working Group will add a statement which refers the user to ISO 16782. This ISO standard describes procedures that can be used to assess the quality of CAMHB.
  - Apparently, some of the ISO AST standards are undergoing revision. The Joint Working Group expressed their support for ISO 16782.
- Suggested Addition to M23S3 Foreword
  - "Mueller-Hinton agar is also used for agar dilution MIC testing and Mueller-Hinton broth (MHB) is the primary medium used for reference broth microdilution (rBMD) MIC testing. MHB from reliable sources must be used when establishing rBMD MIC QC ranges. Similarly, if agar dilution MIC QC ranges are under consideration, MHA that performs reliably must be used. The document "Clinical laboratory testing – criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing, ISO/TS 16782" describes components of MHA and MHB that may impact antimicrobial activity. It also describes tests that can be performed to ensure the concentrations of the essential medium components are correct and the medium will perform reliably. The suggestions in M23S3 can be used to select MHA for reference agar dilution

MIC QC studies, if desired. Details for selecting MHB are not provided in M23S3 and it is suggested that the sponsor refer to ISO/TS 16782 for guidance in selecting MHB that will perform reliably for rBMD MIC QC studies."

SC DISCUSSION (MAIN POINTS)

- EUCAST participated in the discussion and development of the comment.
- What situations is CLSI allowed to refer to an ISO document?
  - CLSI can refer to ISO, and there are already existing places in this document where CLSI refers to ISO documents.

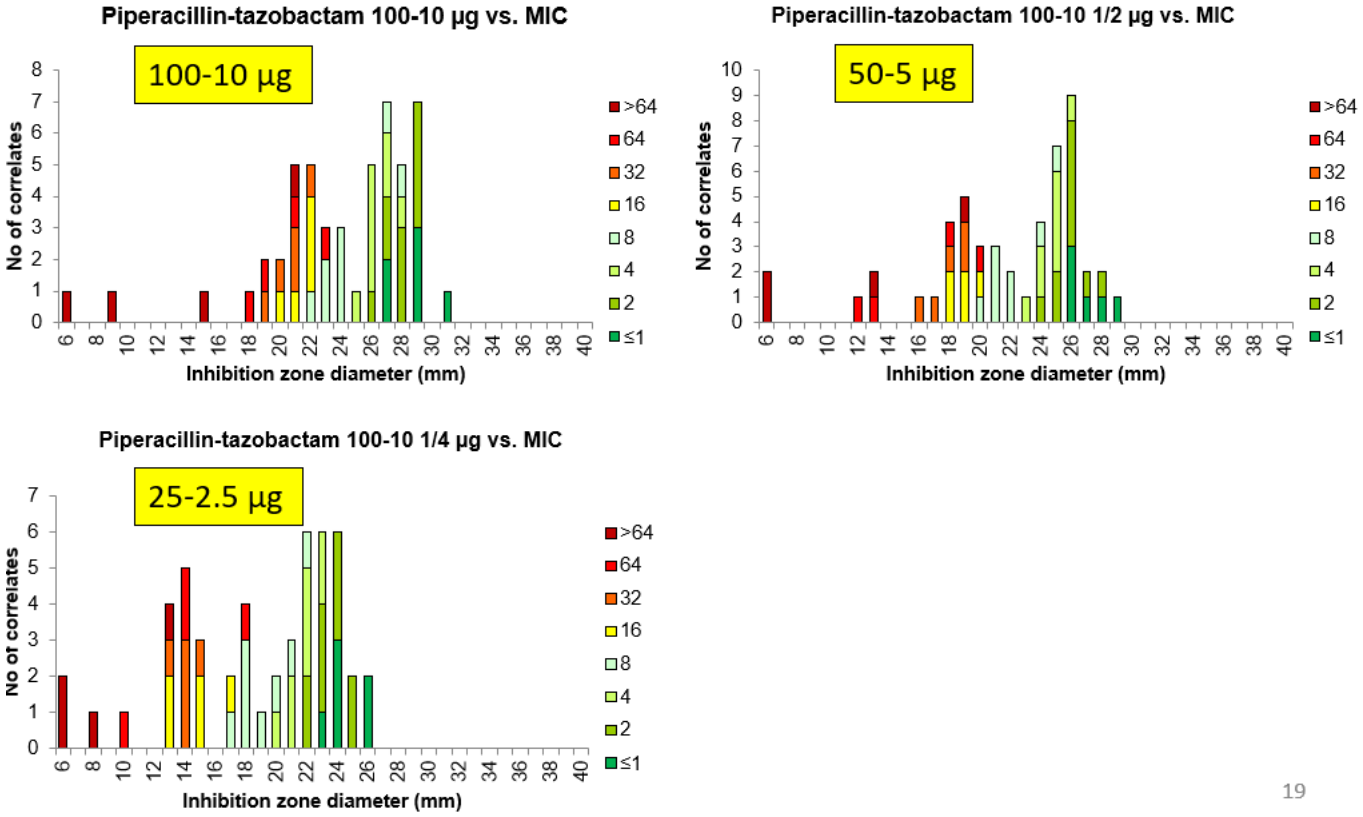
A motion to approve the Forward revisions in CLSI M23S3 as proposed was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

UPDATE ON DISK POTENCY STUDIES

- Process
  - Sub-groups with 2 CLSI and 2 EUCAST members
    - Sub-group decides
    - All Joint WG members will be involved if needed
  - Phase 1
    - 10 disk potencies
    - Few target isolates
  - Phase 2
    - 2-4 disk potencies selected from phase 1
    - 30-60 isolates per species/organism group
- Ceftibuten-xeruborbactam
  - Disk potencies evaluated in phase 2: 5/2 µg, 5/3 µg and 10/1 µg
  - Conclusion
    - Results were acceptable for all three disks tested against 482 Enterobacterales and 24 non-target organisms
    - Among the 3 final disk masses, 5/3 µg seems to perform slightly better
- Cefepime-nacubactam (FEP-NAC) and aztreonam-nacubactam (ATM-NAC)
  - Disk potencies evaluated in phase 2
    - FEP-NAC: 5/10, 5/20, 10/10, 10/20 µg
    - ATM-NAC: 5/10, 5/20, 10/10, 10/20 µg
  - Conclusion
    - Enterobacterales as primary target, *Pseudomonas* as secondary target
    - 10/20 µg selected for both cefepime-nacubactam and aztreonam-nacubactam
- Meropenem-ANT3310
  - Disk potencies evaluated in phase 2: 10/10 and 10/20 µg
  - Conclusion
    - The 10/20 µg was selected for Enterobacterales and *Acinetobacter baumannii*

PIPERACILLIN-TAZOBACTAM STUDIES

- Piperacillin-tazobactam for *E. coli* using CLSI breakpoints and CLSI disk



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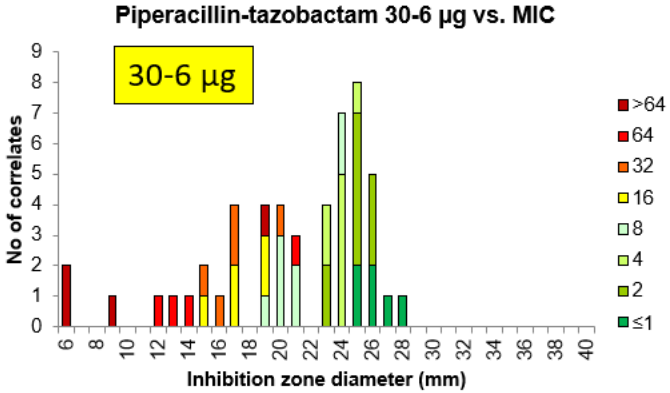
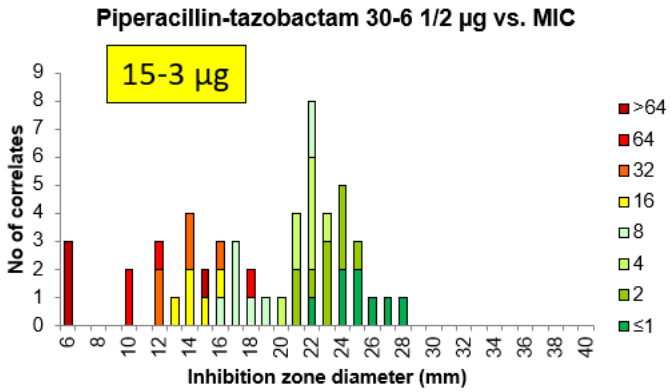
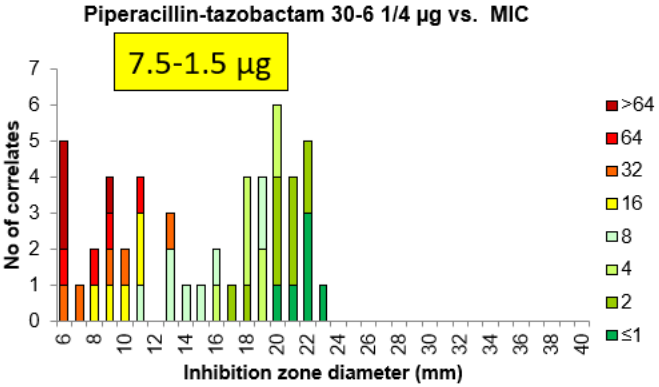
- Piperacillin-tazobactam for *E. coli* using CLSI breakpoints and EUCAST disk







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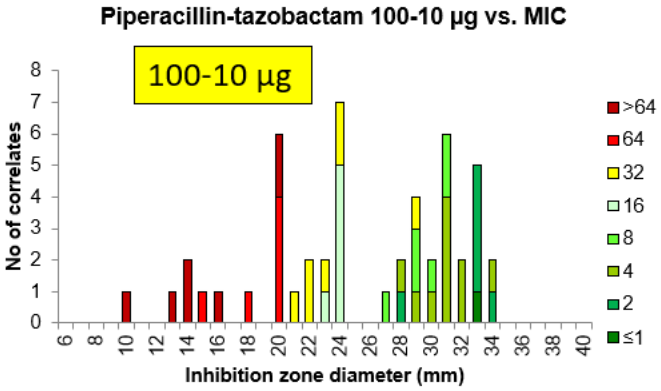
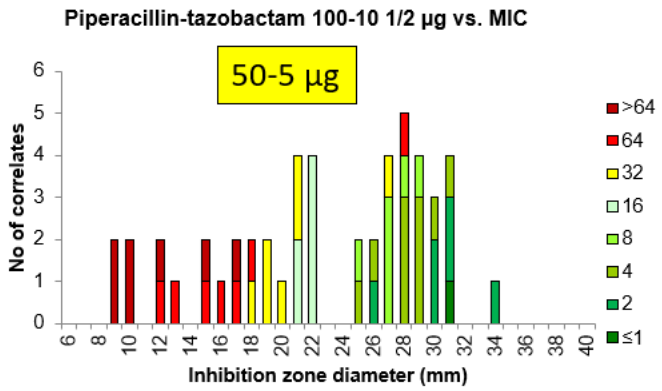
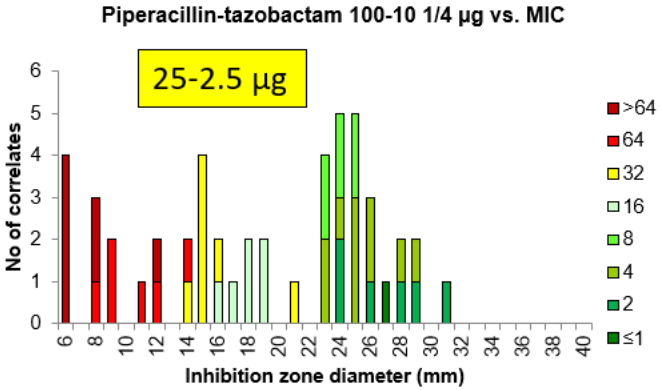
- Piperacillin-tazobactam for *P. aeruginosa* using CLSI breakpoints and CLSI disk







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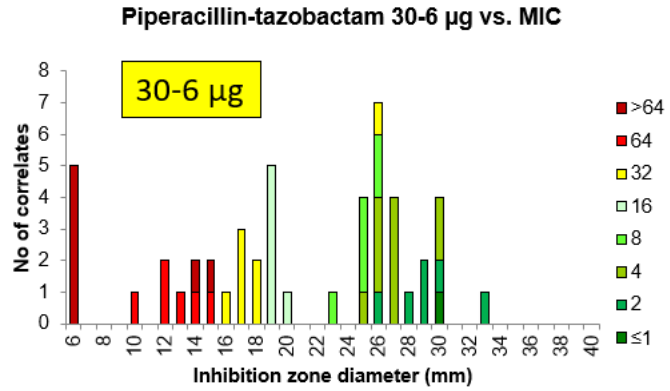
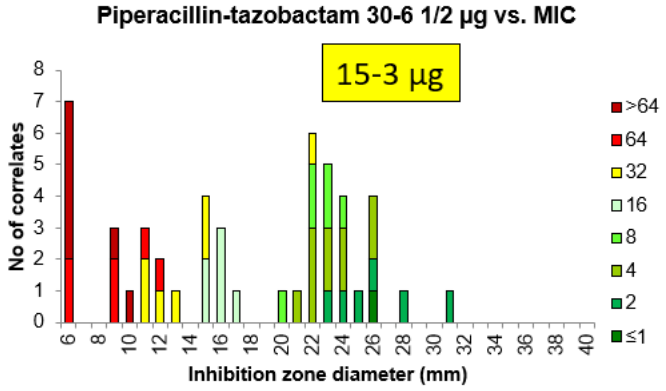
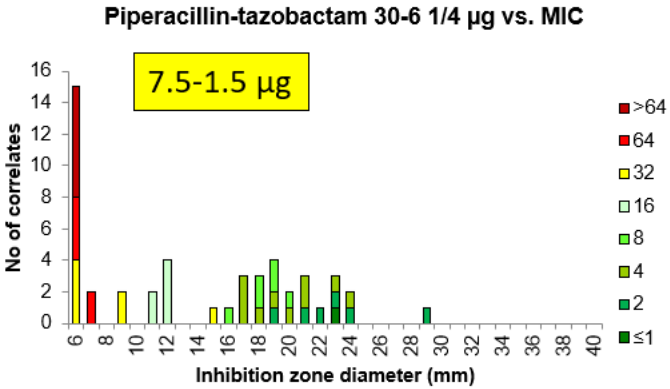
- Piperacillin-tazobactam for *P. aeruginosa* using CLSI breakpoints and EUCAST disk







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SC DISCUSSION (MAIN POINTS)

- Is piperacillin-tazobactam disk diffusion causing false susceptibility? Need to notify laboratories?
  - Laboratories that try to use disks to validate breakpoints have failed because the breakpoints may not be correct.
- With the newly evaluated disk masses this is pushing wild-type disk zone sizes to be less than 15 mm, which CLSI typically does not like to do (note that EUCAST is not concerned if the zone sizes are slightly less than 15 mm for wild-type). Does CLSI need to keep this in mind?
  - The optimal zone size is 15-30 mm which is listed in CLSI documents, and that will be kept in mind when evaluating the data.
- Does CLSI want to further discuss piperacillin-tazobactam disk breakpoints? There are minor errors that trend on the more susceptible side.
  - The risk of laboratories not being able to test piperacillin-tazobactam is a greater risk than the minor errors occurring.
  - Need to make sure CLSI is putting a focus on generating new disk diffusion data to set new disk breakpoints. CLSI has a history of re-analyzing old disk data rather than generating new data, which contributes to issues with test performance.
  - One of the biggest challenges is getting funding for this work.

	○ Disk mass is developed for specific organisms. ○ The majority were not in favor of removing the piperacillin-tazobactam disk diffusion from CLSI M100. ○ There is some disk diffusion data which was collected in the direct from blood cultures bottle studies. ○ Action item: Call for data on piperacillin-tazobactam disks. Joint Working Group will continue to work towards a more optimal disk potency for piperacillin-tazobactam. **UPDATE ON STATISTICAL ANALYSIS** • Can statistical methods be used to identify media and/or disk outliers? • John Turnidge is working on a modified version of RangeFinder, which can identify an outlier laboratory • Pilot testing with EUCAST data looks promising • JMI/Elements and IHMA will provide data from recent CLSI M23 Tier 2 studies and CLSI M23S3 studies for further testing and development of the program **ANTIFUNGAL AST** • CLSI and EUCAST recommend different reference BMD methods for antifungal AST testing and there are significant differences in MICs • Harmonization is unlikely • Suggestion to establish a contact with Maiken Arendrup (Chair of EUCAST AFST Subcommittee) and bring this to the Outreach Working Group for informing laboratories about the differences

9. **ANAEROBE WORKING GROUP (D. CARPENTER)**

CLSI M11 REVISION PROPOSAL UPDATE

- Project proposal submitted to the Expert Panel on Microbiology
  - Request for full revision was not approved
  - Recommendation to reaffirm current version of CLSI M11
    - Concern about inclusion of disk diffusion and gradient strip comments
    - Recommend waiting to revise until disk diffusion work is complete
  - Recommend a journal article to address AST testing for anaerobes
    - Plan to work on a proposal for Clinical Microbiology Reviews
  - Recommend an anaerobe update in the Outreach Working Group newsletter
    - Approved by Outreach Working Group – part of March 2025 issue as a "Hot Topic"
  - Recommend an advisor from the M45 Working Group to help with disk diffusion evaluation
- Anaerobe Working Group Recommendation
  - Motion to reaffirm CLSI M11. WG Vote: 12-0-1-2.

SC DISCUSSION (MAIN POINTS)

- Documents at CLSI must be reviewed every 5 years. A reaffirmation confirms that the information in the document is still relevant, and no updates are needed currently. The document will be reviewed again in 5 years but may be reviewed sooner.
- CLSI M11 is a standard, not a guideline.
- There were concerns of including gradient diffusion strips. Just because there is a gradient diffusion test available; does not mean it is approved for anaerobes.
- There is an issue in the United Kingdom that EUCAST moved to a new medium for anaerobes (FAA medium), but the gradient diffusion strips use brucella agar in the package insert.

A motion to approve the reaffirmation of CLSI M11 was made and seconded. Vote: 12 for, 0 against, 0 abstain, 2 absent (Pass)

EUCAST ANAEROBE DISK DIFFUSION UPDATE

- EUCAST breakpoint tables v15 draft (consultation until mid-December, for publication 01.01.2025)- no changes regarding disk diffusion
- Next phase of anaerobe AST for EUCAST: extending the panel to include additional anaerobes
  - Clostridium – *C. innocuum, C. ramosum* (now *Thomasclavelia ramosa*), *C. tertium, C. septicum*
  - *Cutibacterium avidum*
  - *Fusobacterium nucleatum*
  - GPAC: *Finegoldia magna, Parvimonas micra, Peptostreptococcus anaerobius* and *Peptoniphilus* species
- Anglia Ruskin University expecting isolates for testing early 2025

CLINDAMYCIN TABLE 1J PLACEMENT

- Clindamycin for anaerobes is currently in Tier 1 in Table 1J. Is this the correct placement?

Table 1J. Anaerobes

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ampicillin (gram-positive anaerobes)[a,b] Penicillin (gram-positive anaerobes)[a,b,c]			Ampicillin (gram-negative anaerobes)[a,b] Penicillin (gram-negative anaerobes)[a,b,c]
Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam			
Clindamycin			
Ertapenem Imipenem[d] Meropenem			Imipenem-relebactam[d]
Metronidazole[e]			
			Cefotetan Cefoxitin
			Ceftriaxone
			Moxifloxacin
			Tetracycline

Abbreviation: MDRO, multidrug-resistant organism.

SC DISCUSSION (MAIN POINTS)

- Clindamycin is a common drug, so it does not belong in a Tier 4.
- Data from Mayo Clinic shows that the clindamycin susceptibility for anaerobes is decreasing over the last decade.
- Physicians think that clindamycin is universally active against anaerobes, so laboratories should be testing this drug more to keep physicians aware.
- Anaerobes had Group A or C, which was always test or do not test at all.
- EUCAST is also seeing increasing resistance to clindamycin in anaerobes.
- Are laboratories updating an anaerobe antibiogram?
    - CLSI has found a volunteer to help with an updated anaerobic antibiogram data.
    - Mayo Clinic and IHIMA are the only laboratories performing anaerobe AST by agar dilution.
- Could there be a publication to address the decrease in clindamycin susceptibility?

WORKING GROUP PILOT STUDY OF DISK DIFFUSION METHOD

- Three sites (Mayo, IHMA and Public Health Wales)
    - Completed pilot study
- Methods
    - Disk diffusion - Fastidious Anaerobe Agar (Read at 18 ± 2 hours)
    - Agar Dilution - Brucella Blood Agar and FAA Agar (Read 42-48 hours)
- Organisms
    - 27 clinical isolates (10 from Public Health Wales Challenge Set)
        - *Bacteroides* spp., *Prevotella* spp., *Fusobacterium necrophorum*, *Cutibacterium acnes* and *Clostridioides difficile*
    - 3 QC organisms

- *Bacteroides fragilis* ATCC 25285, *Clostridium perfringens* ATCC 13124, *Clostridium perfringens* DSM 25589 (anaerobic conditions),

- Antibiotics
  - Meropenem (10 µg) – 0.015 – 32 µg/ml
  - Metronidazole (5 µg) – 0.03 - 64 µg/ml
  - Clindamycin (2 µg) – 0.06 – 16 µg/ml
- Anaerobe Environment
  - Chambers (1)
  - Gas Pak (2)
- Categorical Agreement

Meropenem Categorical		Isolate
AD-CLSI vs DD-EUCAST	96.3%	26/27
AD-EUCAST vs DD-EUCAST	92.6%	25/27

Metronidazole Categorical		Isolates
AD-CLSI vs DD-EUCAST	100.0%	22/22
AD-EUCAST vs DD-EUCAST	90.91%	20/22

Clindamycin Categorical		Isolates
AD-CLSI vs DD-EUCAST	92.6%	25/27
AD-EUCAST vs DD-EUCAST	91.7%	22/24

- General Comments
  - Overall performance was acceptable
  - Scatter plot not provided due to low number of values
  - Testing labs used different company for sourcing blood
  - Testing labs used same powder lot for media from one manufacturer
  - One replicate per site, small number of isolates/species and only three sites
  - Reading of zones edge use EUCAST reading guide
    - Clindamycin inner colonies counted following EUCAST guidelines
    - Metronidazole and meropenem inner colonies were not counted (following CLSI guidelines)
- Next Steps
  - Perform analysis of published data to CLSI M23 method requirements
    - Evaluate available QC data in the CLSI format for review
    - Based on the review determine if additional QC data is needed
    - Have an advisor from M45 review and provide feedback before the June meeting

- Anaerobic Working Group Recommendation
  - Motion to accept next steps. WG Vote: 12-0-1-2.

SC DISCUSSION (MAIN POINTS)

- CLSI and EUCAST do not always read inner colonies the same way. The CLSI recommendation is to ignore hazy inner zones and read the outer zone. EUCAST does include reading the inner colonies (ie, reading the inner zone that includes the inner colonies).
- Suggestion to include photos and a reading guide if zones are difficult to read for the study sites.
- Does the method work in the anoxomat system?
  - Yes, there is anoxomat data and it looks good so far.
- EUCAST uses FAA media.
- The intent for anaerobe disk diffusion study is to move forward with FAA agar. The powder for FAA is available in the US, but CLSI needs to check with media manufacturers to see how available it is because availability has varied over the years.

A motion to endorse the review of previously published data and analyze the published data using CLSI M23 methods requirements for disk diffusion QC for anaerobes was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

IN-MEMORIAM – MIKE COX

- September 10th, 2024, Marion (Mike) Ellison Cox passed away at 79 in Morgan Hill, California.
- Founded Anaerobe Systems in 1978
- Active at CLSI – M11 and M56 Anaerobe Documents
- Founding member of the Anaerobe Society of the Americas (ASA) in 1992
- In 2014, he received the ASA Lifetime Achievement Award.
- He has also been recognized for his contribution to science by having a bacterium named after him, *Peptoniphilus coxii*.

10. **QUALITY CONTROL WORKING GROUP (S. CULLEN AND C. PILLAR)**

Please note the QC Working Group votes conducted at the January 2025 meeting did not have government representation.

TIER 2 QC

CONTEZOLID

- Background

Drug: Contezolid (5 µg disk)	**Abbreviation (Glossary II & III): CZD**	**Previous ID**: MRX-1
Solvent (Table 6A): DMSO	**Diluent (Table 6A):** Sterile distilled water or saline	**Preparation (Table 6C combination agents)**: N/A
Route of administration (Glossary II): PO and IV	**Class (Glossary I & II):** Oxazolidinone	**Subclass (Glossary I & II):** NA
Study Report by: IHMA (MIC), Element/JMI (disks)	**Pharma Co**: MicuRx pharmaceuticals	**Control Drugs**: Linezolid (MIC and disk), Tedizolid (disk)
Additional Information (M23 requirements)	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, etc):pH, divalent cations, inoculum size, various media • **ISO/TS 16782 assessment of Tier 2 study materials**: Yes, (pending confirmation from Fluka for MIC)	
Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC):** • Footnote: Read trailing broth microdilution MIC values at the beginning of trailing.	
Discussion	MIC study conducted in 2014 but presented January 2025.	

- Proposed MIC QC Ranges

Drug Name:	Contezolid					Votes:	10/0/3/1 (For, Against, Absent, Abstain)			
QC Strain	**Range**	**% In**	**Mode**	**Dil**	**Shoulder**	**Media Mode**	**Lab Mode**	**M23 Range**	**Range Finder**	**Comments**
***S. aureus* ATCC 29213**	1-4	98.1%	2	3	57.6% @1	2,2,2	3@1, 6@2	1-4, 98.1%, 3 dil	0.5-4, 100%, 4 dil	Some lab variability
***E. faecalis* ATCC 29212**	0.5-4	100%	2	4	68.4% @ 1	2, 2, 1	3@1, 6@2	1-4, 96.7%, 3 dil	0.5-4, 100%, 4 dil	Some media and lab variability
***S. pneumoniae* ATCC 49619**	0.5-2	100%	1	3	46.6% @0.5	1,1,1	3@0.5, 6@1	0.5-2, 100%, 3 dil	0.5-2, 100%, 3 dil	Some lab variability

- Trailing is often observed with oxazolidinones and gram-positive cocci.
- Included readings of 100% and 80% reduction in growth. Data presented/method proposed to use 80% reduction in growth.
- Media tested: Only 2 manufacturers of media were available at time of 2014 study (BBL, Fluka) and two lots of media from BD/BBL were used. Verbal report from JMI that recent data shows mode of 2 for *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 with limited data. Request that pharma present additional QC data from other studies (eg, clinical) to confirm robustness of range set with limited media manufacturer data.
- Add footnotes
  - MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available.
  - QC ranges were established using only two media manufacturers.
  - Read at 80% reduction in growth (use the same footnote as other oxazolidinones)

SC DISCUSSION (MAIN POINTS)

- Typically, data with a tight mode is a 3-dilution range. If there is a shoulder with >60% of mode, then a 4-dilution range is typically considered.

A motion to accept the contezolid MIC QC ranges for *Staphylococcus aureus* ATCC 29213 (1-4 µg/mL), *Enterococcus faecalis* ATCC 29212 (0.5-4 µg/mL), and *Streptococcus pneumoniae* ATCC 49619 (0.5-2 µg/mL) was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

- Proposed Disk Diffusion QC Ranges

Drug Name:	Contezolid (5 µg)				Votes:		11/0/2/1			
QC Strain	Range	% In	Median	Mm	Media	Disk	Labs	Gavan	Range Finder	Comments
***S. aureus* ATCC 25923**	17-23	96.7%	20	7	20,20	3@20,	1@17, 1@19, 3@20, 2@21, 1@22	17-23, 96.7%, 7 mm	16-24, 100%, 9 mm	Lab variability
***S. pneumoniae* ATCC 49619**	17-23	99.4%	20	7	20,20	3@20	1@18, 1@19, 3@20, 2@21, 1@21.5	17-23, 99.4%, 7 mm	17-24, 99.8%, 8 mm	Lab variability

- Media included Remel, BD, Hardy
- Disks included MAST, Oxoid
- Read with reflected light per standard instructions for disks. No issues with reading reported.

A motion to accept the contezolid disk (5 µg) diffusion QC ranges for *Staphylococcus aureus* ATCC 25923 (17-23 mm) and *Streptococcus pneumoniae* ATCC 49619 (17-23 mm) was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

ZOSURABALPIN

- Background

Drug: Zosurabalpin (5 µg)	**Abbreviation (Glossary II & III)**: ZAB	**Previous ID**: RG6006, RO7223280
Solvent (Table 6A): Sterile Distilled Water	**Diluent (Table 6A):** Sterile Distilled Water	**Preparation (Table 6C combination agents)**: N/A
Route of administration (Glossary II): IV	**Class (Glossary I & II):** Peptide	**Subclass (Glossary I & II):** Tethered Macrocyclic
Study Report by: Element (JMI)	**Pharma Co**: F. Hoffmann-La Roche	**Control Drugs**: Cefepime

Additional Information (M23 requirements)	• **Tier 1 Impact Assessment** (stability, inoculum, reading, incubation time, etc): Yes, 22-ROC-01 report • **ISO/TS 16782 assessment of Tier 2 study materials**: Yes
Footnotes:	• **Recommendations for Troubleshooting Guide (Table 4D Disk or 5G MIC):** No.
Discussion	Proposed indication for *Acinetobacter baumannii-calcoaceticus* species complex Suggested text for M100 : Zosurabalpin (ZAB; 5 µg) zone diameter values against *Acinetobacter baumannii* NCTC 13304 are read using the outer zone diameter.

- Proposed Disk Diffusion QC Ranges

Drug Name:	Zosurabalpin (5 µg)				Votes:		11/0/2/1			
QC Strain	Range	% In	Median	Mm	Media	Disk	Labs	Gavan	Range Finder	Comments
Acinetobacter baumannii NCTC 13304	22-28	100%	25	7	25, 26	3@25	1@23, 3@25, 4@26	23-27, 97.7%, 5 mm	22-28, 100%, 7 mm	Slight media difference (only 1mm) 7mm range addresses lab variability and avoid future Tier 3 revision.

- Tier 1 testing conducted with 4 different manufacturers of media (BBL, Remel, Hardy, Carolina) and 3 different manufacturers of disks (Remel, Carolina, Hardy)
- Tier 2 testing
  - Media included Remel, Carolina, Hardy
  - Disks included MAST and Liofilchem
- Suggested footnote: Zosurabalpin (ZAB; 5 µg) zone diameter values against *Acinetobacter baumannii* NCTC 13304 are read using the outer zone diameter.
- Suggest adding picture from presentation.
- Note: Cefepime control results were in the upper half (11-16) of the CLSI range of 6-16 (11 mm range).

SC DISCUSSION (MAIN POINTS)

- Laboratory C was not excluded from the study because it only failed in one of three indicators. CLSI requires two indicators to fail to exclude it.
- Action item for Texts and Tables Working Group: Where will the photo live for reading guidelines? QC Working Group would like the Text and Tables Working Group to find a placement. Text and Tables Working Group indicated it is messy to have a photo in the footnotes but will find an appropriate location for a photo.

A motion to accept the zosurabalpin disk (5 µg) diffusion QC range for *Acinetobacter baumannii* NCTC 13304 (22-28 mm) with a footnote and picture instructing to read the outer zone diameter was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

TIER 3 MIC QC

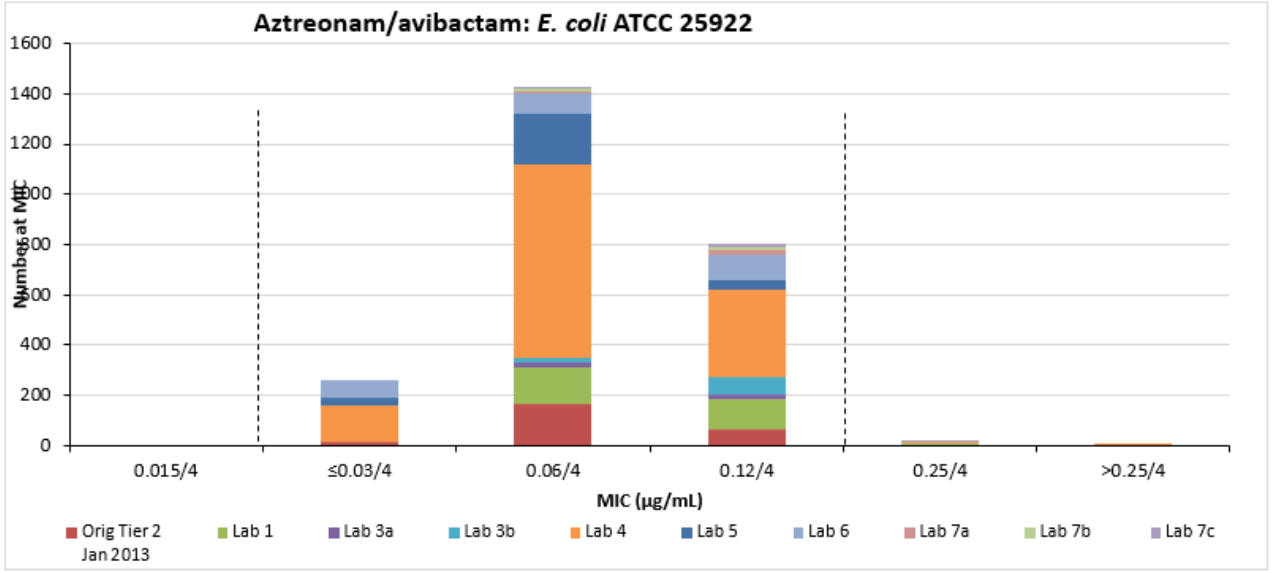
- Proposed range change for:
  - *E. coli* ATCC 25922 with aztreonam/avibactam (*K. pneumoniae* ATCC 700603 is recommended for routine QC)
  - *K. pneumoniae* BAA-1705 with imipenem (for QC integrity)
  - *K. pneumoniae* BAA-2814 with imipenem (for QC integrity)

- Archive the Tier 3 inquiry for *S. pneumoniae* ATCC 49619 with ceftriaxone (the QC range with additional data is acceptable)
- Additional data request for *S. pneumoniae* ATCC 49619 with doxycycline

AZTREONAM AVIBACTAM

- Background

QC Strain (ATCC)	Antimicrobic	Current Range	Action Recommended	Concern/Analysis	Reported
E. coli ATCC 25922	Aztreonam/ avibactam	0.03/4-0.12/4	**QCWG 10/0/4/0** Expand range to 0.03/0/4 - 0.25/4 if EUCAST agrees to also make change to align	Report for shoulder/bimodal distribution with large amount of data at high end of current range. Dec 2023: Additional data added from 3 labs, resulting in 5 total labs with Tier 3 data (n=2158) + Tier 2 (n=237). Tier 3 data has 56% shoulder at 0.12/4, with 3 of 5 labs demonstrating bimodal distributions or a mode at the high end of the range; <1% out of QC high. NOTE: Aztreonam alone was changed from 0.06-0.25 to 0.06-0.5 for the same reason. June 2024: Added supplemental data provided by one lab in Jan 2024 bringing Tier 3 data to n=2198, did not impact prior analysis. Jan 2025: Added supplemental data provided by one lab bringing Tier 3 data to n=2270; that new set was from three sites, one with a mode at 0.12/4 and two bimodal at 0.06/4 and 0.12/4; shoulder at 0.12/4 is 59% of the mode.	21-Jun



- Proposed MIC QC Ranges
  - Current range 0.03/4 - 0.12/4 µg/mL. Tier 3 0.6% out of QC.

- 59% MIC shoulder at 0.12/4 µg/mL. Bimodal or mode at high end of range for majority of laboratories.
- Aztreonam previously expanded from 0.06-0.25 µg/mL to 0.06-0.5 µg/mL, *K. pneumoniae* 700603 is recommended routine QC strain (not *E. coli* ATCC 25922)
- Expand range for aztreonam/avibactam to 0.03/4 - 0.25/4 µg/mL.

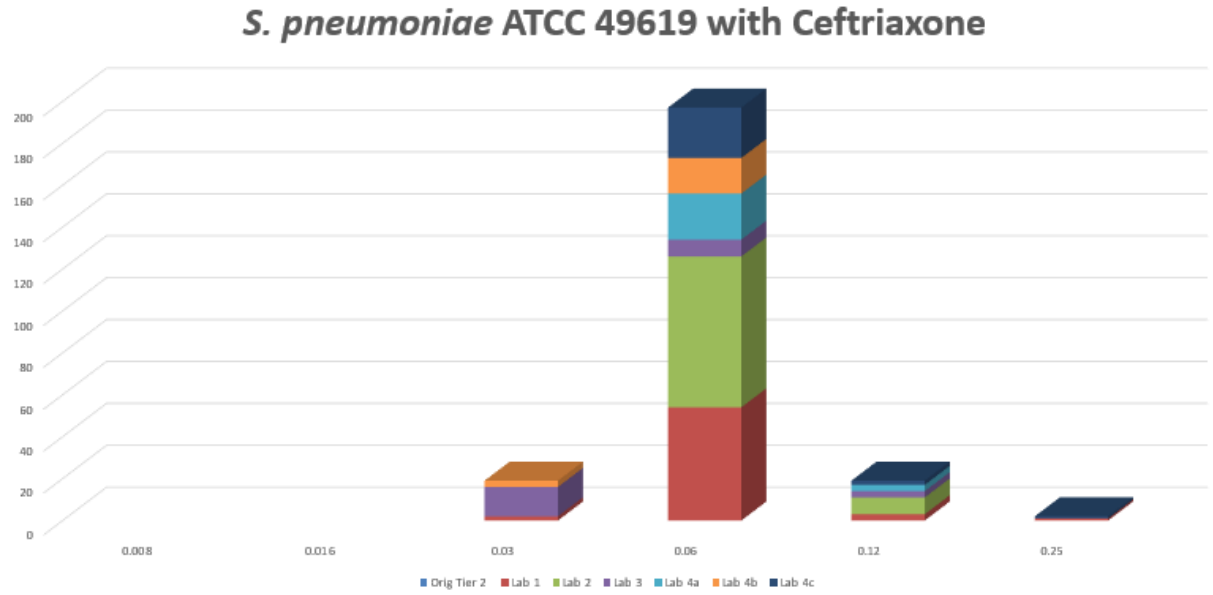
SC DISCUSSION (MAIN POINTS)

- Why expand the range? The Minnesota Department of Health runs two controls of the *E. coli* ATCC 25922 every time to ensure one is in, because the value is often high.
- Why does the avibactam, in combination with aztreonam, bring down the QC Range? CLSI needs to clarify if there is a ß-lactamase.
  - Element/JMI states the ß-lactamase is not active.
  - Avibactam does have some independent binding to PBP2 and aztreonam binds to PBP3, so often see that targeting two PBPs can lower the MICs 1 to 2 dilutions even in isolates without ß-lactamases.
- EUCAST would not change the aztreonam/avibactam QC range as proposed. It needs more discussion.
- Action item: QC Working Group to work with EUCAST to determine if the MIC range for aztreonam-avibactam should be changed.

CEFTRIAXONE

- Background

S. pneumoniae ATCC 49619	Ceftriaxone	0.03-0.12	**QCWG 10/0/4/0** Data supports current range. No change/archive.	Signal reported from one lab that there may be an issue with MICs frequently observed at the upper end of the range. Data based on freeze-dried panels, need reference data to determine whether this is in fact a signal for the reference method. Dec 2023: data from 3 labs using reference method added, two show strong mode in middle of range with no appreciable shoulder and <1% out of QC high. June 2024: Added supplemental data provided by one lab in Jan 2024 bringing Tier 3 data to n=2198, did not impact prior analysis. Jan 2025: Added supplemental data provided by one lab bringing Tier 3 data to n=237; that new set was from three sites, all with modes at the middle of the current range without any shoulders. <1% out of QC.	22-Nov



- Added supplemental data provided by one laboratory bringing Tier 3 data to n=237; that new set was from three sites, all with modes at the middle of the current range without any shoulders. <1% out of QC.

- Proposed to archive Tier 3 inquiry/investigation of *S. pneumoniae* ATCC 49619 MIC QC range with ceftriaxone

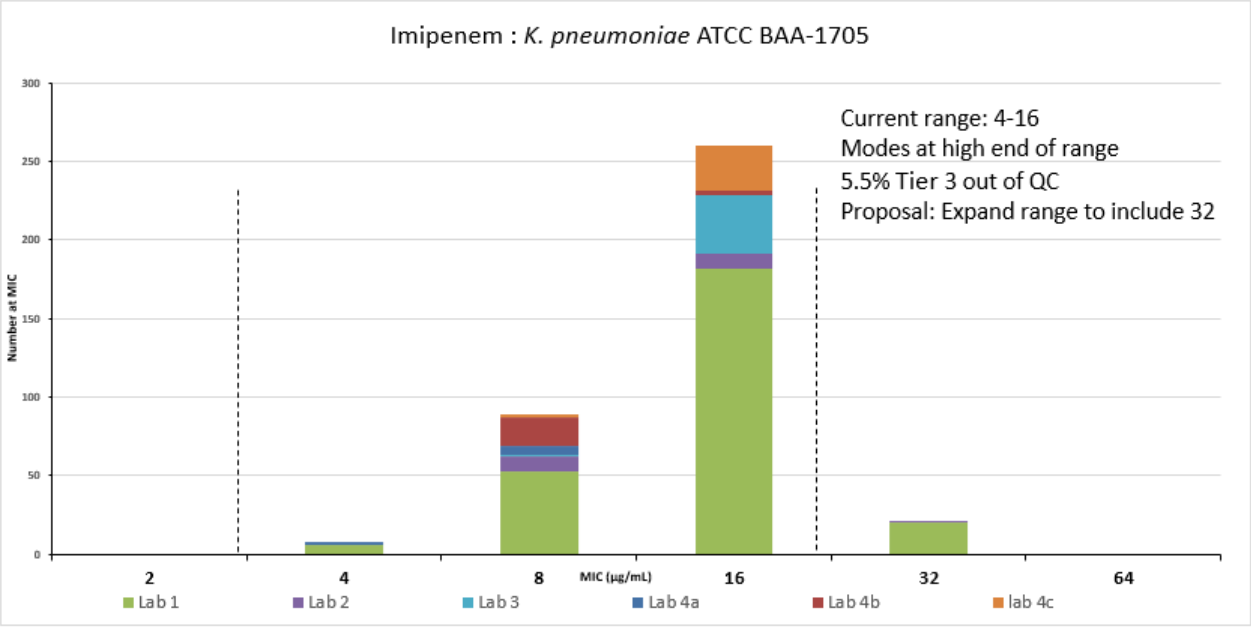
A motion to archive the Tier 3 inquiry for the *Streptococcus pneumoniae* ATCC 49619 ceftriaxone MIC QC range (0.03-0.12 µg/mL) was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

NOTE: After the January 2025 meeting, it was clarified by the QC Working Group that the intention was for the *Streptococcus pneumoniae* ATCC 49619 ceftriaxone MIC QC range (0.03-0.12 µg/mL) to be retain in CLSI M100.

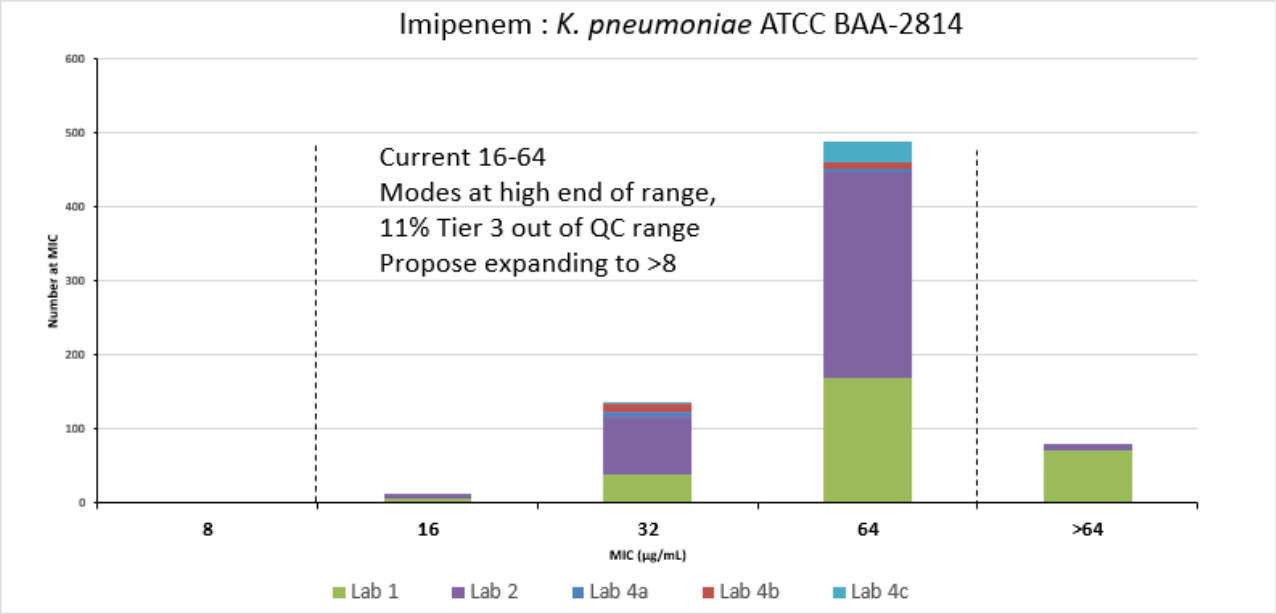
IMIPENEM

- Background

QC Strain (ATCC)	Antimicrobic	Current Range	Action Recommended	Concern/Analysis	Reported
K. pneumoniae BAA-1705	Imipenem	4-16	QCWG 10/0/4/0 Expand range to 4-32.	Signal from recent Tier 2 study showed a mode at 16 (70% of total results) and out of QC results at 32 (6.7%). Dec 2023: Data from 2 additional labs added, resulting in data from 3 labs (n=318), all show similar results with a bimodal distribution or the mode at the high end of the range. Jan 2025: Data from an additional lab brings us to 4 labs (n=378) and supports expanding range to 4-32. Majority of results are at 16 which is the high end of the range.	23-Jan
K. pneumoniae BAA-2814	Imipenem	16-64	QCWG 10/0/4/0 Revised to >8	Signal from Tier 2 study showed a mode at 64 (67% of total results) and out of QC results at 128 (24.8%). Dec 2023: Data from 1 additional lab added, resulting in data from 2 labs (n=656). Results from both labs are similar with the mode at the high end of the range and out of QC results high. Need data from more than 2 labs to take action. Jan 2025: Data from an additional lab brings us to 3 labs (n=716) and supports expanding range to 16-128 or >8. The vast majority of results are at 64, the high end of the current range.	23-Jan



- Majority of imipenem *K. pneumoniae* ATCC BAA-1705 results are at 16 µg/mL, which is the high end of the current range.
- Used for QC integrity.
- Data now includes 4 laboratories (n=378) and supports expanding range from 4-16 µg/mL to 4-32 µg/mL.



- Majority of imipenem *K. pneumoniae* ATCC BAA-2814 results are at 64 µg/mL, which is the high end of the current range.
- Used for QC integrity.
- With additional laboratory, data are sufficient (3 laboratories, n=716) and supports amending range to >8 µg/mL.

- Proposed MIC QC Ranges
  - Imipenem *K. pneumoniae* ATCC BAA-1705 MIC QC range of 4-32 µg/mL.
  - Imipenem *K. pneumoniae* ATCC BAA-2814 MIC QC range of >8 µg/mL.

SC DISCUSSION (MAIN POINTS)

- There was discussion on if the imipenem *K. pneumoniae* ATCC BAA-2814 MIC QC range should be >8 µg/mL or ≥16 µg/mL.
- Saying >8 µg/mL or ≥16 µg/mL are the same and CLSI M100 has similar scenarios written both ways.

A motion to accept the imipenem MIC QC range for *Klebsiella pneumoniae* ATCC BAA-1705 (4-32 µg/mL) was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

A motion to accept the imipenem MIC QC range for *Klebsiella pneumoniae* ATCC BAA-2814 (≥16 µg/mL) was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

TIER 3 MIC QC DATA REQUEST

S. pneumoniae ATCC 49619	Doxycycline	0.016-0.12	No new data provided for review.	Signal from EDL 5 lab dried panel study where nearly 70% of results tested at 0.12, the high end of the range; requesting frozen reference method data to see if further monitoring or adjustment is warranted Jun 2024: no reference data submitted	23-Jun

TIER 3 DISK DIFFUSION QC

- Additional data requested for the following
  - Spectinomycin 100 µg for *N. gonorrhoeae* ATCC 49226
  - Ceftibuten 30 µg for *E. coli* NCTC 13353

QC Strain (ATCC)	Antimicrobic	Current Range	Action Recmd	Concern	Update	Date Reported
N. gonorrhoeae ATCC 49226	Spectinomycin 100 µg	23-29	Continue to monitor until June 2025. Request additional data.	QC study out high	January 2025: No additional data. June 2022: Observations in gentamicin QC study, especially with one lab and media	June-22
E. coli NCTC 13353	Ceftibuten 30 µg		Continue to monitor until January 2027. Request additional data. Find Tier 2 data	Zone diameters in the lower part of range and out of range	January 2025: No additional data.	Jan-24

AST ROUTINE USER QC IMPROVEMENTS

- Four educational documents and tools
  - Rationale for Updated CLSI Guidance AST QC for the Clinical Laboratory
  - Quick Guide for Updated CLSI Guidance AST QC - QC Frequency and Selection of QC Strains
  - IQCP MIC Tool (to select QC strains based on antimicrobial agents/dilutions tested)
  - IQCP Example with Updated CLSI Guidance AST QC
- Also Refer to CLSI M100 35th Ed for specific guidance
  - Appendix I: Selection of QC Strains and QC Testing Frequency
  - Table 2's: QC Recommendation Boxes
- QC Working Group January 2025 Virtual Meeting Summary

- Rationale for Updated CLSI Guidance AST QC for the Clinical Laboratory
  - Provides background and justification for change.
- Quick Guide for Updated CLSI Guidance AST QC: QC Frequency and Selection of QC Strains
  - Highlights concepts and steps to take. Refers to CLSI M100 Appendix I for details.
- IQCP Example with Updated CLSI Guidance AST QC
  - Updated sections for QA (training, proficiency) and QC (key indicators of deterioration)
  - Includes examples of how QC might be reduced
    - EXAMPLE A: Testing of selected ATCC QC strains twice monthly (approximating the 1st and the 15th of each month) for routine QC testing.
    - EXAMPLE B: Testing of selected ATCC QC strains monthly (approximating the 1ST of each month) for routine QC testing.
    - EXAMPLE C: Weekly testing of at least 1 selected ATCC QC strain on one of 4 instruments weekly, rotating instruments each week for routine QC testing so that all ATCC QC organisms and instruments are tested at least monthly. Appropriate QC strains to select will be indicated in laboratory QC procedures.
    - NOTE: Testing protocols other than those listed above could be established if supported by the laboratory's IQCP/QCP
- IQCP MIC Tool
  - Uses similar approach as breakpoint tools. Helps to identify which QC strains for QC Plan.
    - Provide examples for MIC (more challenging since many QC strains have ranges off-scale). Can also use for disk diffusion (but simpler since no off-scale QC ranges).
    - Provides for user MIC QC ranges for gram-positive and gram-negative QC strains (extracted from CLSI M100)
    - User adds drugs/dilutions on AST method.
    - Follow CLSI M100 Appendix I to assess value of QC strains and select QC strains for lot/shipment vs routine QC. (eg, critical indicators, on-scale, historical QC issues).
  - Suggested improvements:
    - Add flow chart and additional explanations (from verbal presentation).
    - Add ranges for all gram-negative and gram-positive QC strains. Update annually.
    - Include tab for example and tab for user to define QC plan for their AST device.
    - Optional column for user to add manufacturer's recommended QC.
    - For ß-lactamase/ß-lactamase inhibitors, use green highlights showing QC strains recommended for routine QC (instead of on-scale).
    - Create short video to walk through tool (like breakpoint tool video).
  - Instructions:
    - User adds drugs/dilutions on AST device.
    - Can use filters in Excel to display drugs on device.
    - Identify recommended QC strains for routine testing for ß-lactamase/ß-lactamase inhibitors (green).
    - Determine which MICs are on-scale (yellow).
    - Identify key indicators (using CLSI M100 Appendix I and laboratory's historical data).
    - Based on this information, identify QC strains to include in QC Plan (IQCP).
  - Example recommendations:
    - Lot/Shipment QC:

- *E. coli 25922*: optional for agents not addressed by other QC strains (eg, cefazolin)
- *P. aeruginosa* 27853: detects carbapenem (imipenem best indicator) and other drug deterioration; on-scale for multiple drugs
- *E. coli* 35218 or *K. pneumoniae* 700603: include to detect deterioration (clavulanate good indicator) for multiple ß-lactamase/ß-lactamase inhibitors
- *S. aureus* 29213 and *E. faecalis* 29212: optional for on-scale for multiple drugs; only consider if QC ranges are provided in commercial AST Instructions for Use
- *E. faecalis* 29212: optional, to confirm media quality for trimethoprim-sulfamethoxazole

- Notes:
  - Consider historical QC performance to determine if optional QC strains needed.
  - Additional strains needed if additional agents included on AST device
  - *K. pneumoniae* ATCC BAA-1705 and/or *K. pneumoniae* BAA-2814: select for imipenem-relebactam and meropenem-vaborbactam
  - *A. baumannii* NCTC 13304: select for sulbactam-durlobactam
- Routine QC (minimum):
  - *P. aeruginosa* ATCC 27853: select to detect deterioration of carbapenem (imipenem good indicator)
  - *E. coli* ATCC 35218 and/or *K. pneumoniae* ATCC 700603: include to detect deterioration (clavulanate good indicator)
  - Select other QC strains as indicated based on historical data from IQCP review
  - Additional strains needed if additional agents included on AST device
    - *K. pneumoniae* ATCC BAA-1705 and/or *K. pneumoniae* BAA 2814: include if testing imipenem-relebactam and meropenem-vaborbactam
    - *A. baumannii* NCTC 13304: include if testing sulbactam-durlobactam

SC DISCUSSION (MAIN POINTS)

- Recommendation to have laboratory bench techs provide feedback.
- Notify the manufacturers of the new CLSI IQCP MIC tool
- CLSI M02 and CLSI M07 were revised in March 2024. This new QC guidance could affect these document updates in the future.
- Is CLSI going to make a tool for disk diffusion?
  - Currently, do not have examples for disk diffusion, but it would be helpful in the future
- CLSI M100 Appendix I addresses different system components (open vs closed systems).
- There was a concern that the IQCP MIC tool Excel file could be overwhelming for a technologist. CLSI plans to include a video on how to use it.
- There is not a requirement for laboratories to use the IQCP MIC tool. It is an optional resource for laboratories.
- What is the timeline?
  - The AST Subcommittee will need to vote on the finalized educational documents and tool prior to posting on the website.
- Action Item: Provide example on what happens when QC does not pass in this streamlined approach? Does the laboratory go back to more frequent QC?
- Action item: A future topic to look at what to do when QC fails.

ß-LACTAMASE/ß-LACTAMASE INHIBITOR COMBINATION QC

- ß-lactamase/ß-lactamase inhibitors QC recommendations
  - CLSI will continue to recommend best QC strains for routine testing to minimize number of QC strains for users to test.

- EUCAST currently recommends a susceptible and resistant QC strain. They will consider options to indicate when a susceptible strain is recommended (eg, manufacturer vs user)

- Best practice to include 3 lots of media when doing MIC Tier 3 for control drug (if room on panel) to help monitor and reevaluate if needed for Tier 3.
- Continue to use Tier 3 process to monitor/reassess when control drug from a Tier 2 study has high % out of range or results at extremes of QC range.
- Consider adding information on BLIs if they have activity against QC strain (to assist with setting range and confirm alignment of range for ß-lactamase and ß-lactamase inhibitors
- Compile list of differences between CLSI and EUCAST QC ranges. Review and triage in June (eg, no action, assess with Tier 3)

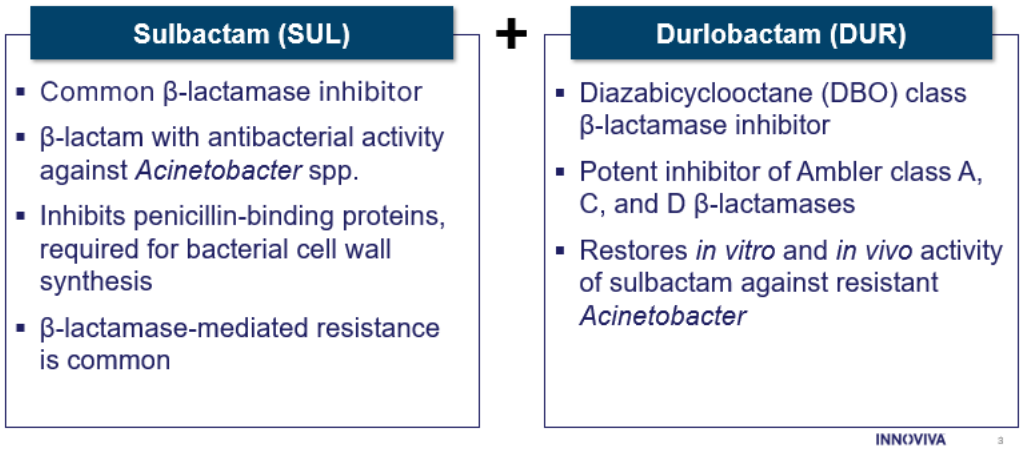
MISCELLANEOUS

- Anaerobe Working Group: Proposed approach to establish disk QC ranges with FAA media
  - Propose use of Tier 3 to set QC ranges for disk diffusion as allowed by CLSI M23 6th Ed if:
    - data includes sufficient sample size and
    - parameters adequately represented and can be evaluated separately (eg, media manufacturers, laboratories, disks manufacturers)
- QC Working Group agreed with approach
- Anaerobe Working Group to provide examples for consideration in June 2025

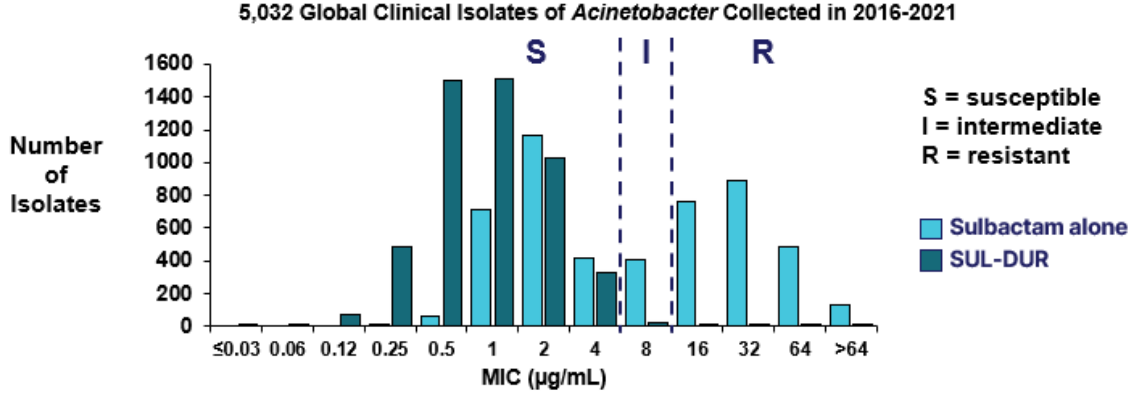
11. **TABLE 1 PLACEMENT OF SULBACTAM-DURLOBACTAM (S. MCLEOD)**

Dr. McLeod presented on the Table 1 placement of sulbactam-durlobactam for *Acinetobacter* spp.

- XACDURO® (Sulbactam-Durlobactam) Indication and Dose
  - Approved by US FDA May 2023
  - Indicated in adults (≥ 18 years) for the treatment of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia caused by susceptible strains of *Acinetobacter baumannii-calcoaceticus* complex
  - Dose: 1.0 g sulbactam / 1.0 g durlobactam
  - Schedule: q6h administered as 3-hour IV infusion
  - Dose adjustments recommended in patients with CL_{CR} < 45 mL/min or ≥ 130 mL/min
- Sulbactam-Durlobactam is a Targeted Antibiotic



- Durlobactam Restores Sulbactam Activity Against Global Clinical *Acinetobacter* Isolates



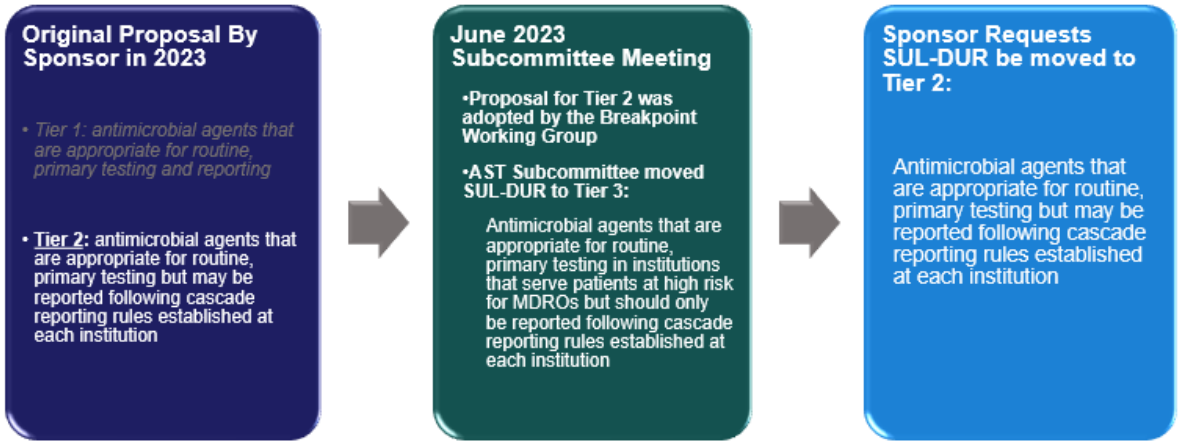
- Sulbactam alone MIC90 = 64 µg/mL
- SUL-DUR MIC_{90} = 2/4 µg/mL
- 98.3% of isolates had SUL-DUR MIC ≤ 4/4 µg/mL

- Interpretive Criteria for Sulbactam-Durlobactam against *Acinetobacter* spp.
  - Approved by FDA May 2023 and CLSI June 2023

	MIC (µg/mL)			Zone Diameter (mm)		
Pathogen	S	I	R	S	I	R
Acinetobacter spp.	≤4/4	8/4	≥16/4	≥17	14-16	≤13

S = susceptible; I = intermediate; R = resistant

- Susceptibility Testing for Sulbactam-Durlobactam (SUD)
  - Validation set of 30 *A. baumannii* isolates available through Laboratory Specialists, Inc.
  - Cleared devices:
    - Disk diffusion tests available (sulbactam/durlobactam 10/10 µg)
      - HardyDisk (Mast) – SUD20
      - Oxoid (ThermoFisher)
    - Gradient strip
      - Etest (BioMérieux)
    - Dried Panels
      - Sensititre (ThermoFisher)
- CLSI M100 Table 1B-2. *Acinetobacter* spp. Placement



- IDSA 2024 Guidance on the Treatment of Antimicrobial Resistant Gram-Negative Infections
  - Tamma PD et al. (2024) *Clinical Infectious Diseases*, ciae403, https://doi.org/10.1093/cid/ciae403
  - Treatment of carbapenem-resistant *Acinetobacter baumannii* (CRAB):
    - Sulbactam-durlobactam, in combination with meropenem or imipenem-cilastatin, was added as the preferred agent for the treatment of CRAB infections.
    - High-dose ampicillin-sulbactam in combination with at least one other agent has been changed from a preferred to an alternative regimen if sulbactam-durlobactam is not available.
  - Change in Table 1B-2 placement is justified based on new IDSA Guidance
- Proposed Table Placement for Sulbactam-Durlobactam in CLSI M100 Table 1B-2. *Acinetobacter* spp.

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinical request if antimicrobial agents in other tiers are not optimal because of various factors
Ampicillin-sulbactam			
Ceftazidime	Imipenem Meropenem	Cefiderocol	
Cefepime			
Ciprofloxacin Levofloxacin			
Gentamycin Tobramycin	Amikacin		
	Piperacillin-tazobactam **Sulbactam-durlobactam**		
	Trimethoprim-sulfamethoxazole		
	Minocycline		Doxycycline
		~~Sulbactam-durlobactam~~	
			Cefotaxime Ceftriaxone
			Colistin or polymyxin B

SC DISCUSSION (MAIN POINTS)

- Does the FDA STIC website specify the *Acinetobacter baumannii calcoaceticus* complex?
  - FDA recognizes the CLSI M100 for sulbactam-durlobactam.
- Does a comment need to be added to state only for *Acinetobacter baumannii calcoaceticus* complex?
  - The clinical indication is only for the complex, so a footnote would be appropriate to clarify.
  - It is hard for laboratories to definitively identify the complex.
  - Cefiderocol is for the complex, so that comment could be applied here.
  - Should state the breakpoint should apply to only the *Acinetobacter baumannii calcoaceticus* complex. That way it does not discourage laboratories from doing an MIC.
  - Do not have any signal that there is a problem or failure at the ECV for non-complex isolates.
  - The table is for *Acinetobacter* spp. Consensus was to leave as is without a complex comment.
- Piperacillin-tazobactam and sulbactam-durlobactam should be in separate cells on the table because the mechanism is different.
- *Acinetobacter* is different from organisms like CRE. There is high resistance and not many good drug options.
- Tier 3 was specifically designed for these cases. The presentation is missing the full definition of Tier 2 and Tier 3. Nothing about Tier 3 prohibits laboratories from primary testing. Need to reconsider the placement of other β-lactam combination agents if sulbactam-durlobactam is moved to Tier 2.
- The methods to testing sulbactam-durlobactam are currently manual. Therefore, laboratories that use commercial systems cannot easily test sulbactam-durlobactam as a primary drug.
- Disagreements were heard for the argument that if sulbactam-durlobactam is moved to Tier 2 in the table then the other β-lactam combination agents will need to be moved.

- Table 1 as written is for the US. Data from the ARLN have 25,000 CRAB isolates tested for the main carbapenemases and 2% were NDM. Sulbactam-durlobactam does not work for NDMs; thus, this drug is a reasonable empiric choice while waiting on antimicrobial susceptibility testing.
- Laboratories have limited resources and drug availability. Many laboratories share panels for *P. aeruginosa* and *Acinetobacter*.
- Consider what is the difference is between cefiderocol vs sulbactam-durlobactam from a clinical position.
- IDSA and the WHO have asked companies to develop drugs, so companies are going to lose interest in developing drugs in the future if they are not going to be tested routinely.
- 50% of *Acinetobacte*r were CRAB (26% in US). The rates of CRAB are high outside of the US (80%). MALDI-TOF does a good job differentiating the complex from non-complex organisms. The non-complex organisms are typically highly susceptible to multiple drugs.
- Why is this not listed as a cascade reporting for carbapenem resistant isolates?
    - Initially the data was not there, and the sponsor did not want a reflex testing from carbapenem resistance.
    - Would the sponsor want to move sulbactam-durlobactam to cascade after carbapenem?
        - Yes, the sponsor is open to cascading against carbapenems.
- Sulbactam-durlobactam is only available in US and China.
- There was concern that small community hospital laboratories would not know to consider this drug.
- Makes sense to move the sulbactam-durlobactam in the table to be right next to cefiderocol.
    - Would sulbactam-durlobactam reflex from ampicillin-sulbactam or carbapenems?
    - Sulbactam-durlobactam is most like ampicillin-sulbactam. Suggest putting in the same row as ampicillin-sulbactam.
    - Sulbactam-durlobactam is preferred over cefiderocol. Suggest being placed above cefiderocol.

A motion to move sulbactam-durlobactam to Tier 3 cascading off carbapenems in Table 1B-2 (*Acinetobacter* spp.) was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

- New Table 1B-2 Mockup from Text and Tables Working Group

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ampicillin-sulbactam			
Ceftazidime	Imipenem Meropenem	Sulbactam-durlobactam Cefiderocol	
Cefepime			
Ciprofloxacin Levofloxacin			
Gentamicin Tobramycin	Amikacin		
	Piperacillin-tazobactam		
	Trimethoprim-sulfamethoxazole		
	Minocycline	~~Sulbactam-durlobactam~~	
			Cefotaxime Ceftriaxone
			Colistin or polymyxin B

12.	**ADJOURNMENT** Dr. Mathers thanked the participants for their attention. The meeting was adjourned at 12:00 PM Eastern Standard (US) time.

2025 JANUARY AST MEETING SUMMARY MINUTES PLENARY 2: Monday, 27 January 1:00 PM – 5:30 PM Eastern Standard Time (US)	
#	**Description**
1.	**OPENING** Dr. Mathers opened the meeting at 1:00 PM Eastern Standard (US) time.

2. **BREAKPOINTS WORKING GROUP (N. NARAYANAN AND M. SATLIN)**

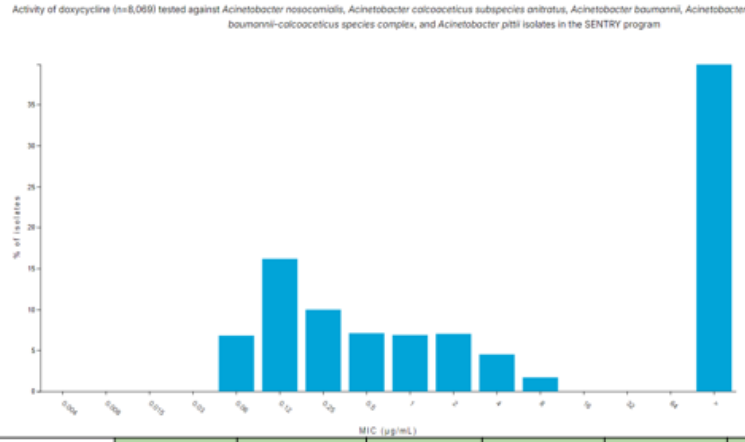
Please note the Breakpoints Working Group votes conducted at the January 2025 meeting did not have government representation.

DOXYCYCLINE BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Tetracycline Breakpoints

	Previous/old breakpoints (2024 M100-ED34)			Revised/current breakpoints (during June 2024 meeting)		
	S	**I**	**R**	**S**	**I**	**R**
Minocycline	≤ 4	8	≥ 16	≤ 1	2	≥ 4
Doxycycline	**≤ 4**	**8**	**≥ 16**	**Archived; under review**		
Tetracycline (urine)	≤ 4	8	≥ 16	Archived; under review		

- Doxycycline vs *Acinetobacter baumannii* MIC distributions
  - JMI (SENTRY)

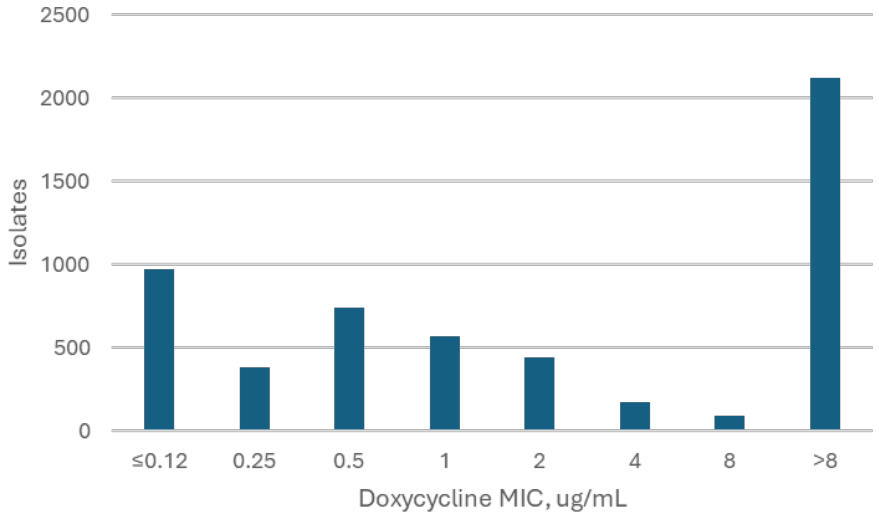


JMI (SENTRY)
Doxycycline MIC distribution
8,069 *Acinetobacter baumannii* complex isolates with doxycycline MICs

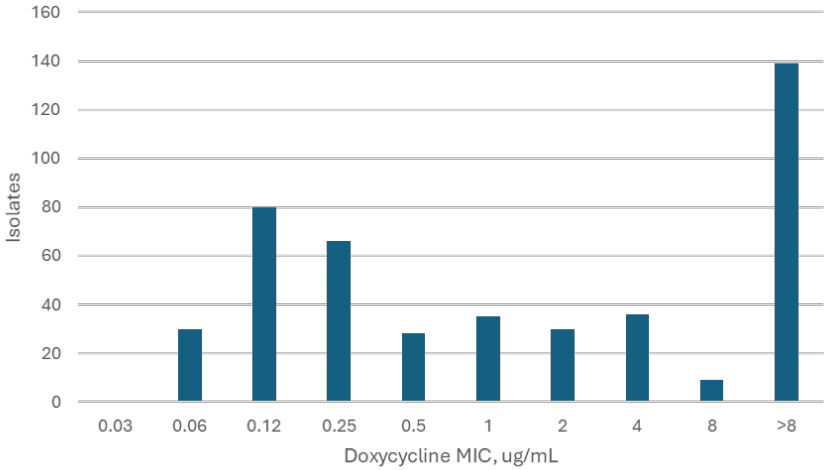
Doxycycline MIC, µg/mL	≤ 0.06	0.12	0.25	0.5	1	2	4	8	> 8
n (cumulative %)	548 (6.8)	1,311 (23.0)	803 (33.0)	569 (40.0)	556 (46.9)	561 (53.95)	362 (58.4)	141 (60.1)	3,218

https://sentry-mvp.jmilabs.com/. Accessed 13 May 2024.

- Castanheira et al. Clin Infect Dis 2014; PMID: 25371512
  - 5477 isolates from a worldwide surveillance program (2007-2011)
  - Tested by CLSI reference BMD at JMI
  - MIC distributions truncated on lower end

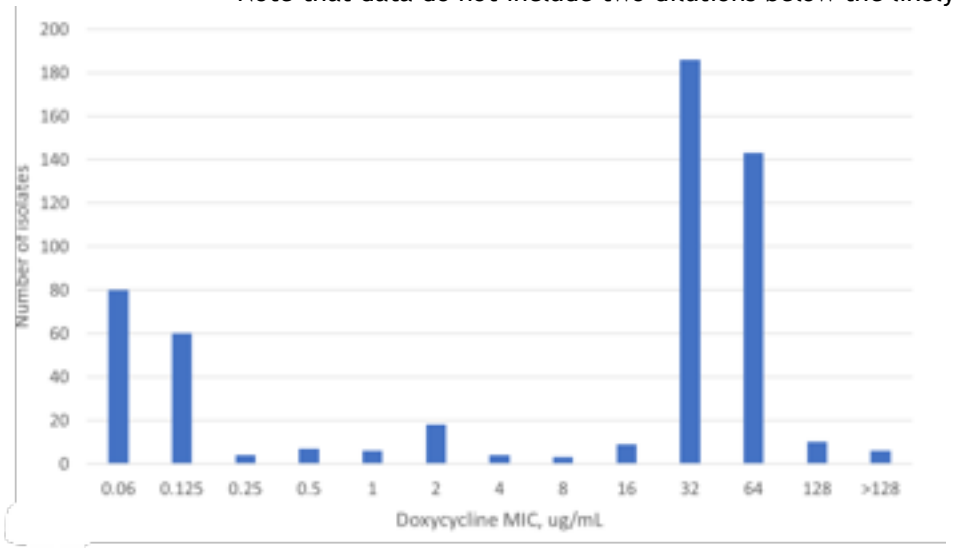


- Huband et al. Antimicrob Agents Chemother 2020. PMID: 32071042
  - 531 recent (most from 2018) clinical isolates collected from patients in 34 countries
  - Tested by CLSI reference BMD at JMI

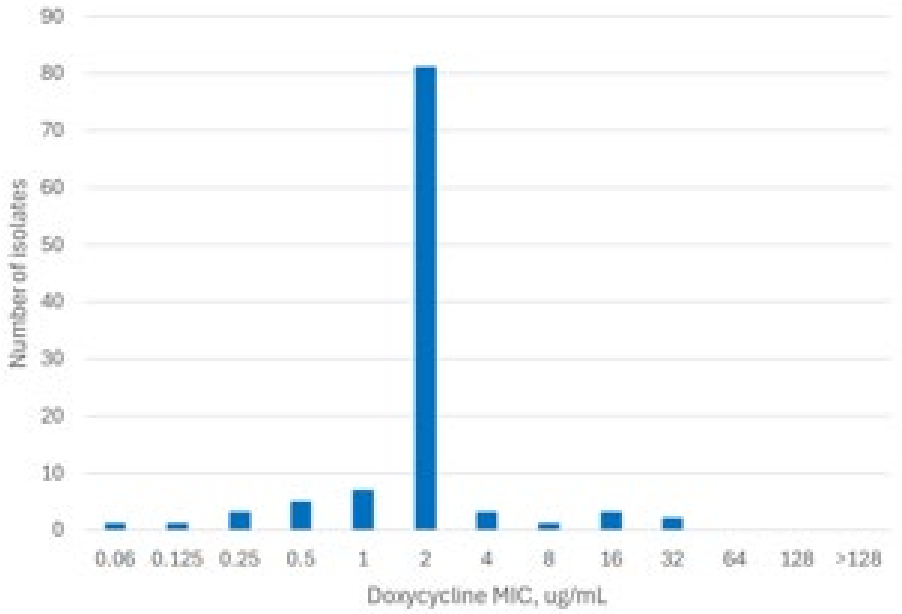


- Hans R, Ding L, et al. Microbiol Spectr 2022; 10:e0147121 (536 *A. baumannii* isolates from China, 2018-2019)

- Data were provided to the *Acinetobacter* AHWG upon request, courtesy of Fupin Hu.
- 536 clinical *A. baumannii* isolates from 20 hospitals in 13 provinces and cities in China between January 2018 and December 2019
- Tested by CLSI reference BMD
- Note that data do not include two dilutions below the likely wild-type mode (ie, truncated on the low end)



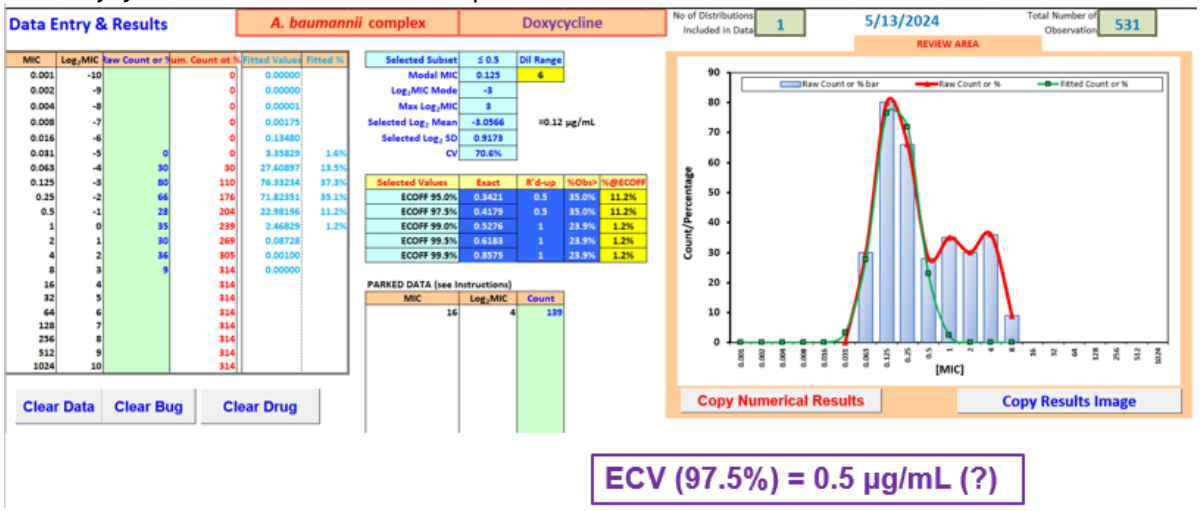
- Mezzatesta ML et al. Ann Clin Microbiol Antimicrob 2008; 7: doi:10.1186/1476-0711-7-4
  - 107 clinical isolates collected from 9 Italian hospitals (2002-2003)
  - Tested by CLSI reference BMD in one laboratory
  - Note the very different shape of this MIC distribution compared to the other datasets (appear to be very few wild-type isolates included)



- No data were available from the EUCAST website, ATLAS (Pfizer), Shionogi dataset (AMR Register/VivLi), or the IHMA dataset from the sulbactam-durlobactam studies
- ECOFF Finder
  - It was not possible to officially run ECOFF Finder using any of the identified data sets
  - Truncated distributions on the low end
    - An MIC distribution should test at least two dilutions below the modal MIC of wild-type isolates to be acceptable for aggregation in ECOFF Finder
    - Most of the available data sets do not meet this criterion
    - Including data sets truncated on the low end can lead to misleading answers from ECOFF Finder
    - The "least truncated" distribution is the one published in the JMI AAC paper
  - Large JMI dataset lumps together multiple species within the complex
  - Should technically only set an ECV for a single species, but okay to include multiple closely related species that are difficult to readily separate
  - ECOFF Finder attempts including various data sources to generate variable results

Data source(s) included	n	ECV 95.0%	ECV 97.5%	ECV 99.0%	ECV 99.5%	ECV 99.9%
JMI MVP	8,069	0.5	0.5	1	1	1
JMI CID (2007-2011 global)	5,477	0.25	0.25	0.25	0.25	0.25
JMI AAC (2018 global)	531	0.5	0.5	1	1	1
China (2018-2019)	536	0.12	0.12	0.12	0.12	0.12
Italy (2002-2003)	107	2	4	4	4	4
JMI MVP + China (not weighted, JMI >>50% data)	8,605	0.5	0.5	1	1	1
JMI MVP + China + Italy (not weighted, JMI >>50% data)	8,712	0.5	0.5	1	1	1
JMI AAC + China (no weighting needed)	989	0.25	0.25	0.5	0.5	0.5
JMI AAC + China + Italy (no weighting needed)	1,096	0.25	0.25	0.5	0.5	0.5

- Doxycycline ECV based on AAC JMI publication data



ECV (97.5%) = 0.5 µg/mL (?)

  - Doxycycline and *A. baumannii* ECV Summary
    - Imperfect data sets to establish a formal doxycycline ECV per CLSI M23 criteria

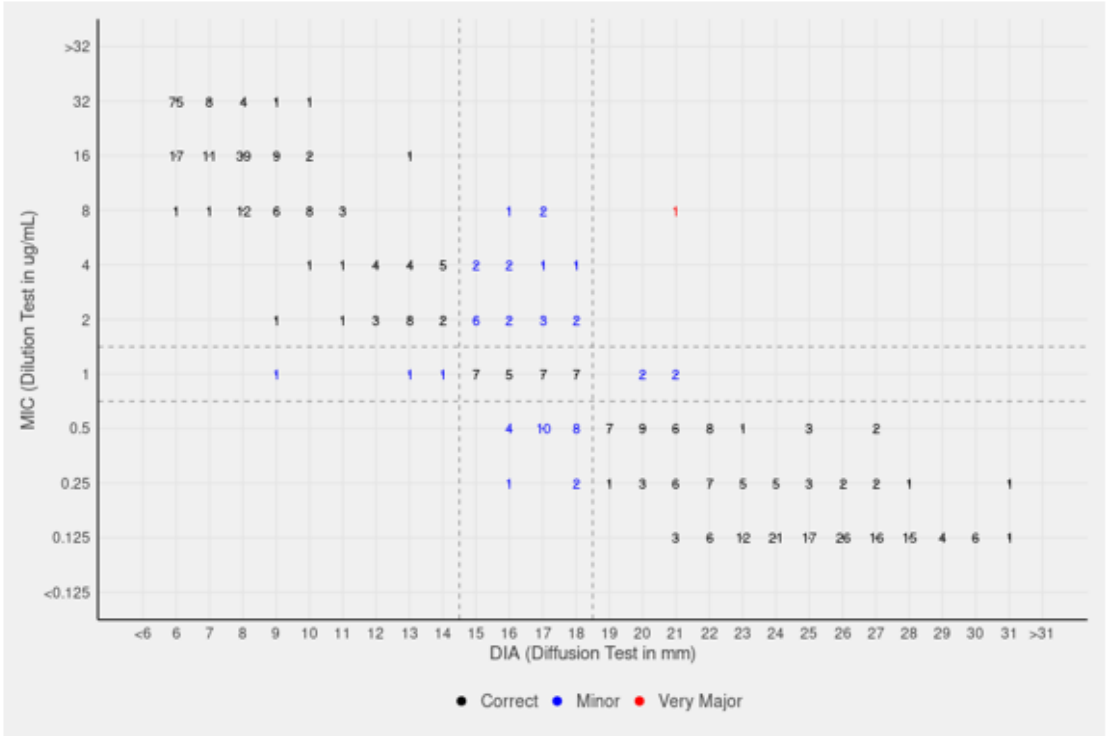
- MIC distributions are truncated on lower end
- Formal ECV required 3 laboratories and data from Italian laboratory difficult to include

▪ *Acinetobacter* AHWG estimated a doxycycline ECV of 0.5 µg/mL for *A. baumannii* complex based on data from JMI AAC paper (least truncated, isolates from 34 countries)

- Doxycycline vs *Acinetobacter baumannii* PK-PD
  - Tetracyclines PK and dosing comparisons
    - PK is variable with ranges throughout the older literature
  - Doxycycline vs minocycline PK high variability
    - AUC after 200-400 mg TID ~ 100-340 mg · h/L
    - ~fAUC 24-82 mg · h/L
  - Doxycycline vs minocycline dosing
    - Max dose for doxycycline per package insert is 200 mg daily
    - Up to 300 mg of doxycline daily for syphilis
    - Minocycline label allows up to 200 mg twice daily (400 mg/day) which is PK that new breakpoint is based upon
  - Doxycycline PK/PD target for *A. baumannii*
    - Do not exist.
    - There are no contemporary PK/PD studies for doxycycline.
      - The fAUC/MIC target is unknown
      - The fAUC/MIC target cannot be assumed to be the same as minocycline
  - Cannot assume same PK-PD target for all tetracyclines
  - Safety/tolerability data for high-dose (200 mg twice daily) doxycycline?
    - Minimal safety and tolerability data from small case series for patients treated with doxycycline for neurosyphilis
- Doxycycline clinical outcomes
  - IDSA guidance on CRAB treatment
    - Doxycycline not mentioned as an option for the treatment of carbapenem-resistant *A. baumannii* in IDSA guidance document
  - Clinical data: Tetracyclines for MDR *A. baumannii*
    - Falagas et al. Int J Antimicrob Agents 2014. PMID: 25801348
      - Very few patients treated with doxycycline for *A. baumannii* infection in this review
    - Tuon et al. Braz J Microbiol 2023. PMID: 37278889
      - Largest study of doxycycline for *A. baumannii*
      - Retrospective single-center study of 100 hospitalized adults with *A. baumannii* infections from 2017-2020 in Brazil who received ≥3 days of oral doxycycline
      - Isolates identified by MALDI-TOF Vitek MS
      - Confirmed susceptibility to doxycycline
        - 14 isolates underwent doxycycline MIC testing by BMD
        - MIC range: 0.25-2 µg/mL
        - 94% of isolates carbapenem-resistant (all CRAB had bla_{OXA-23} and bla_{OXA-51})
      - 100 patients: 62 pulmonary, 28 SSTI
        - 23 admitted to ICU, 6 required vasopressors

- 31% polymicrobial; combo therapy received in 38% but all rec'd doxy as the only active drug
- Oral doxy Rx median 8 days (range: 6-13)
  - Dosing not specified but paper states that "doxycycline may be prescribed as 100 mg PO q12h or 200 mg q24h"
- Death rate at 14 and 28 days of follow-up were 9% and 14%, respectively
- Many limitations, no outcomes by MIC, no comparator group
  - Emailed authors for outcomes by MIC but no response to date

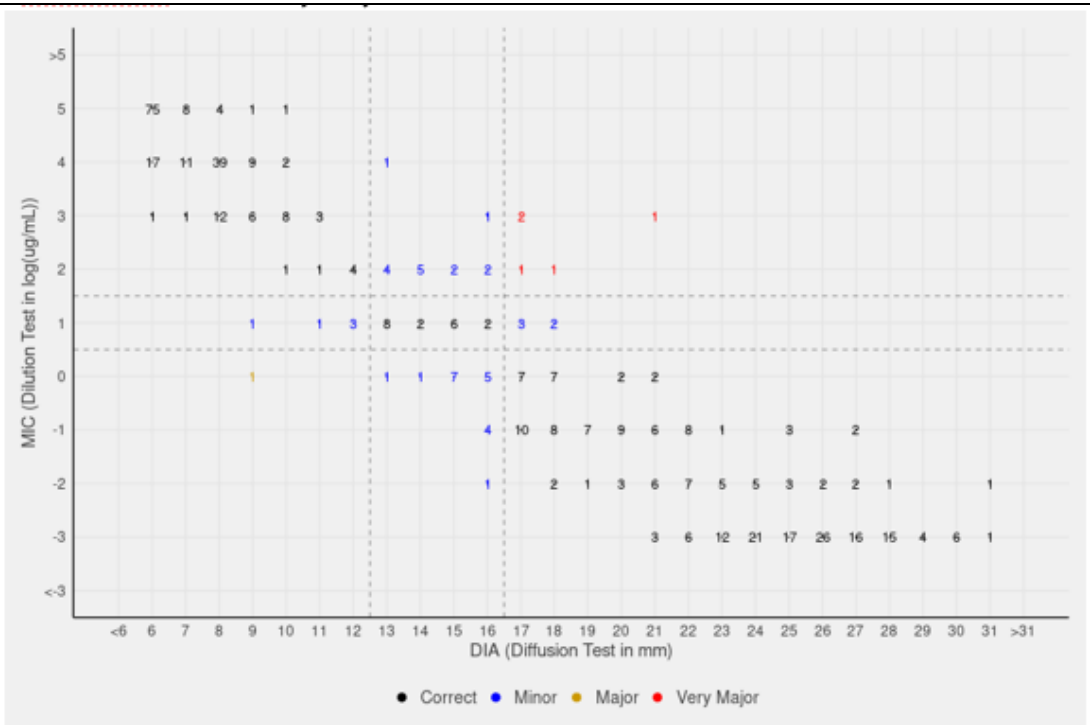
- Doxycycline disk diffusion vs MIC correlates
  - dBETs for 0.5, 1, 2 Susceptible, Intermediate, and Resistant (SIR)



S ≥ 19, I 15-18; R ≤ 14

	VME	ME	mE
I+2	0.4%		4.0%
I +/- 1	0.0%	0.0%	35.3%
I - 2		0.0%	1.8%

  - dBETs for 1, 2, 4 SIR



S ≥ 17, I 13-16; R ≤ 12

	VME	ME	mE
I+2	1.5%		1.0%
I +/- 1	2.5%	0.0%	45.7%
I - 2		0.0%	2.2%

- Acinetobacter AHWG Discussion and Recommendations
  - Vote 1: Remove doxycycline breakpoint entirely
    - No specific PK/PD data
    - Sparse clinical data
    - Unclear how commonly used – but of use outside US?
    - AHWG Vote: 7-0-0-1
  - Vote 2: Move breakpoint to ≤1 S; 2 I; ≥4 R
    - Matches minocycline
    - Fits an ECV of 1 (good for the entire *Acinetobacter* population)
    - Disk correlates are ok (similar errors to minocycline)
    - AHWG Vote: 0-7-0-0
  - Vote 3: Move breakpoints to ≤0.5 S; 1 I; ≥2 R
    - Takes into consideration that doxy fAUC is lower than minocycline and 400 mg daily likely needed to match PK
    - Fits an ECV of 0.5 (ok for entire population, good for non-CRAB)
    - Disk correlates look good
    - AHWG Vote: 4-3-0-0

- Breakpoint Working Group Discussion and Recommendation
  - How were original breakpoints set?
    - Extrapolated from breakpoints for other organisms (no *Acinetobacter*-specific data)
  - If there was strong clinical data that doxycycline works for *A. baumannii* infections and an ECV, could set breakpoint at ECV
    - Clinical data: one retrospective, non-comparative, single-center study
    - ECV: does not meet CLSI M23 criteria
  - If remove breakpoint, need to communicate why
  - Motion to remove doxycycline breakpoints for *A. baumannii* (currently archived). WG Vote: 9-1-0-3 (no government representation).
    - No vote because no clinical signal that doxycycline does not work.

SC DISCUSSION (MAIN POINTS)

- Doxycycline is not used as part of routine care, so a lack of data does not necessarily mean clinical failure.
- Doxycycline is also not commonly used outside of the US.
- Consider if there is a minocycline shortage, would providers want doxycycline?
  - Consensus was that doxycycline is not commonly used if there is a minocycline shortage.

A motion to remove the doxycycline breakpoints for *Acinetobacter* spp. was made and seconded. Vote: 12 for, 1 against, 0 abstain, 1 absent (Pass)

Against Vote Reasoning:

- There is no data stating doxycycline should not be used.

MINOCYCLINE BREAKPOINTS FOR *ACINETOBACTER* SPP.

- Concerns of only having minocycline breakpoint for the tetracyclines vs *A. baumannii*
  - Some automated panels have tetracycline and doxycycline, but not minocycline
  - Other panels may not have minocycline dilutions down to 1 µg/mL
  - There are FDA-cleared disks and a gradient diffusion test for minocycline
  - Can tetracycline and/or doxycycline AST results be used to predict minocycline AST?
- Previous prediction comment
  - CLSI M100-Ed34 (before *Acinetobacter* spp. minocycline breakpoints were lowered)

> "Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both."

- Precedents for using drugs without breakpoints of their own to predict susceptibility to other drugs
  - For agents with established clinical efficacy and considering site of infection and appropriate dosing, methicillin (oxacillin)-susceptible staphylococci can be considered susceptible to:
    - ß-lactam combination agents (amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam)

- Oral cephems…
- Parenteral cephems…
- Carbapenems…

- Isolates of pneumococci with oxacillin zone sizes of ≥ 20 mm are susceptible (MIC ≤ 0.06 µg/mL) to penicillin. Penicillin and cefotaxime, ceftriaxone, or meropenem MICs should be determined for isolates with oxacillin zone diameters ≤ 19 mm, because zones ≤ 19 mm occur with penicillin-resistant, -intermediate, or certain-susceptible strains. For isolates with oxacillin zones ≤ 19 mm, do not report penicillin as resistant without performing a penicillin MIC test.
  - For non-meningitis isolates…an oxacillin zone ≥ 20 mm can predict susceptibility to the following β-lactams….

- Draft comment for consideration
  - Adapted from the *S. pneumoniae* oxacillin comment (with the addition of "also considered" from the existing tetracycline/doxycycline/minocycline comment)
  - Do not have a sufficiently robust data set for disk diffusion to comment on tetracycline or doxycycline zone of inhibitions that predict minocycline susceptibility; however, if laboratories are going to test by disk, they can test minocycline directly
  - Limited the draft comment to *A. baumannii* complex because that is where there is data and is the clinical scenario in which minocycline AST results are most likely to be needed

Isolates of *A. baumannii* complex with tetracycline MICs ≤ 4 µg/mL or doxycycline MICs ≤ 1 µg/mL are also considered susceptible (MIC ≤ 1 µg/mL) to minocycline. Minocycline should be tested directly for isolates with tetracycline MICs ≥ 8 µg/mL or doxycycline MICs ≥ 2 µg/mL because such MICs occur with minocycline-resistant, -intermediate, or certain -susceptible strains. For isolates with tetracycline MICs ≥ 8 µg/mL or doxycycline MICs ≥ 2 µg/mL, do not report minocycline as resistant without directly testing minocycline.

- Breakpoint Working Group Discussion and Recommendation
  - No data available for comparison of tetracycline and doxycycline MIC values with minocycline MIC values using automated systems
  - If breakpoints removed (not just archived), device manufacturers would be inclined to remove tetracycline and doxycycline from panels for *A. baumannii* with software
  - Clinicians: minocycline frequently used for *A. baumannii* and preferred the comment to help
  - Tetracycline urine breakpoint also archived: no data submitted to agenda book -> will review and present in June (likely less data than with doxycycline)
  - Motion to accept the proposed minocycline comment for *Acinetobacter baumannii*. WG Vote: 10-0-0-3 (no government representation).

SC DISCUSSION (MAIN POINTS)

- Question asked that if it matters if a breakpoint is "archived" vs "removed" for manufacturers.
  - Removing minocycline breakpoints indicates to manufacturers to remove doxycycline.
  - CLSI confirmed that archival and removal are the same. Breakpoints are removed from CLSI M100 and get placed into the online archival document.
  - Most laboratories do not know the online archive exists and it is difficult for people to know if something is being reviewed or if something has been truly removed.

- Texts and Tables Working Group could in the future clarify breakpoints that are under revision and being evaluated vs breakpoints being permanently removed.
- Manufacturers would still be able to report MICs and laboratories could use the MIC as a surrogate moving forward.

- The main message is laboratories should test minocycline, and the proposed comment provides an opportunity for those who have not yet brought on minocycline testing.
- Consider adding a preface sentence to the comment saying: "If laboratories do not have minocycline testing available…"
- Laboratories often do not have minocycline on their primary panels.
- Do both tetracycline and doxycycline need to be included?
  - Both are needed. Tetracycline is most common on panels, but doxycycline is better.
- Wording suggestion to say "are considered susceptible" instead of "are also susceptible"
- Suggestion for the final sentence to be standardized with the *S. pneumoniae* comment.
- Action Item: CLSI needs to make a decision about tetracycline breakpoints for A. baumannii for the June 2025 meeting.
- Action Item: *Acinetobacter* AHWG determine the tier placement in Tables 1.

A motion to accept the proposed minocycline comment for *Acinetobacter baumannii* without the last sentence was made and seconded. Vote: 8 for, 5 against, 0 abstain, 1 absent (Fail)

Against Vote Reasoning:
- CLSI should harmonize with other comments.
- Keep the last sentence.

A motion to accept the proposed minocycline comment for *Acinetobacter baumannii* with wordsmithing from Text and Tables Working Group was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

- Comment provided by Text and Tables Working Group following the meeting: ***It is recommended to test minocycline directly when it is available. If minocycline cannot be tested, isolates with doxycycline MICs ≤1 µg/mL or tetracycline MICs ≤4 µg/mL are considered susceptible to minocycline. Isolates with doxycycline MICs ≥2 µg/mL or tetracycline MICs ≥8 µg/mL should be tested against minocycline if that result is needed for treatment.***

GONOCOCCUS (GC) AD HOC WORKING GROUP REPORT

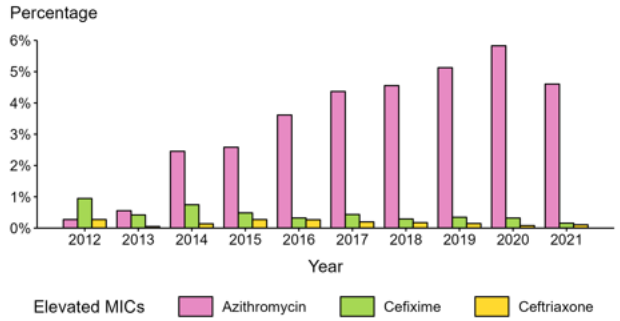
- AHWG Charge
  - Reassess breakpoints for extended-spectrum cephalosporins

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL		
		S	I	R	S	I	R
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)							
Ceftriaxone	30 µg	≥ 35	–	–	≤ 0.25	–	–
CEPHEMS (ORAL)							
Cefixime	5 µg	≥ 31	–	–	≤ 0.25	–	–

- CLSI M23 criteria for reassessment:
  - Susceptible only breakpoints do not enable reporting of emerging resistance
- Timeline:
  - Provide a progress report at the January 2025 CLSI AST Subcommittee Meeting
  - Propose breakpoints at the June 2025 CLSI AST Subcommittee Meeting

- US Treatment Guidelines for *N. gonorrhoeae*
  - Update to CDC's Treatment Guidelines for Gonococcal Infection, 2020 in MMWR
    - In Dec 2020, CDC released updated guidance for the treatment of uncomplicated gonorrhea in adolescents and adults, ahead of the overall STI treatment guidelines release. This guideline was incorporated in the 2021 guidelines later on.
    - For uncomplicated gonorrhea, the ceftriaxone dose was increased to 500 mg IM once while dual therapy was no longer recommended.
    - There was a note added to increase the dose for higher weight persons.
    - When chlamydia is not ruled out, chlamydial treatment is also recommended.
    - Additional guidance for gonorrhea for alternative treatment, where ceftriaxone is not available, or for pharyngeal gonorrhea.
- Resistance data from the US gonococcal isolate surveillance program

Neisseria gonorrhoeae — Percentage of Isolates with Elevated Minimum Inhibitory Concentrations (MICs) to Azithromycin, Cefixime, and Ceftriaxone, Gonococcal Isolate Surveillance Project (GISP), 2012–2021



- **NOTE:** Elevated MICs = Azithromycin: ≥ 2 µg/mL; Cefixime: ≥ 0.25 µg/mL; Ceftriaxone: ≥ 0.125 µg/mL

- International treatment guidelines
  - WHO: Updated recommendations for the treatment of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Treponema pallidum* (syphilis), and new recommendations on syphilis testing and partner services
- Breakpoint Issues for Prioritized CLSI Action
  - MIC Breakpoints (Agar dilution; µg/mL)

	CLSI			FDA			EUCAST			Problem
	S	I	R	S	I	R	S	I	R	
Ceftriaxone	≤0.25	none	none	≤0.25	none	none	≤0.125	none	>0.125	No CLSI R defined. EUCAST is different.
Cefixime	≤0.25	none	none	≤0.25	none	none	≤0.125	none	>0.125	No CLSI R defined. EUCAST is different.

    - Prioritized actions for this committee: CLSI MIC and disk diffusion breakpoint assessment
- Gonococcal Antimicrobial Surveillance Program (GASP-Canada)
    - Voluntary, passive surveillance system monitoring antimicrobial susceptibilities of *N. gonorrhoeae* since mid-80's - approximately 3000 isolates submitted annually – database includes 60K isolates
        - Isolates are sent for testing if they are resistant to at least one antimicrobial tested, approaching resistance to key antimicrobials, or have not been tested for AMR
        - Includes isolates from all body sites (urogenital, rectal, pharyngeal)
    - Antimicrobial susceptibilities were tested using CLSI agar dilution method
        - All isolates received prior to November 2022
        - After November 2022, only if met the following criteria:
            - Azithromycin, ceftriaxone, cefixime whole genome sequencing predicted MICs approaching and over the resistant breakpoint
            - Isolates that failed whole genome sequencing
    - ECVs were calculated using the CLSI ECOFF Finder v21, with an ECOFF value of 97.5%
- Data from National Library of Medicine (NML) and WHO presented on ceftriaxone and cefixime
- CDC Gonococcal Isolate Surveillance Program (GISP) Data
    - MIC Distribution Surveillance Data from US GISP = > 15,000 isolates from 2014 – 2016
    - AST by agar dilution, from multiple sites and laboratories
    - Genetic Marker Analysis on a subset of GISP isolates (723 isolates, selection biased towards higher MICs)
- Ceftriaxone Treatment Failures by MIC: Case reports

Site of Infection	Sex	Age	Year	Country	CRO MIC	Treatment	Outcome	PMID
Pharynx	Female	20s	2024	Vietnam	0.5 mg/L	CRO 1 g + DOX	TOC Culture Positive, failed AZM 2 g (TOC NAAT positive, culture negative), cleared with ETP 1 g IV x1	39417254
Genital/Rectal	Female	30s	2018	Spain	1.0 mg/L	CRO 1 g	Genital cleared, rectal persisted (TOC culture positive), failed GEN 240 mg + AZM 2 g (symptoms persisted), cleared with ETP 1 g IV x3	30862336
Genital/Pharynx	Male	50s	2018	Thailand	1.0 mg/L	CRO 1 g + DOX	Genital cleared, pharyngeal persisted (TOC culture positive), failed SPC (TOC culture positive), cleared with ETP 1 g IV x3	29991383
Genital/Pharynx	Male	20s	2015	Japan	0.25 mg/L	CRO 500 mg + AZM 1g	Genital cleared, pharyngeal persisted (TOC culture positive), cleared with CRO 1 g + AZM 2 g	27332921
Genital	Male	50s	2022	Australia	0.25 mg/L	CRO 500 mg + AZM 1.5g	TOC NAAT Positive (culture negative) at 2 weeks, cleared with augmentin 1 g BID x 7d	35713023
Pharynx††	Female	20s	2009	Japan	2.0 mg/L	CRO 1 g	TOC NAAT Positive at 2 weeks, cleared with repeat CRO 1 g	21192886

- Key Questions to Answer Next
  - Should the CLSI ceftriaxone susceptible breakpoint be lowered to 0.125 µg/mL?
  - If so, is a MIC of 0.25 µg/mL best classified as intermediate or resistant?
  - PK/PD data can help.
    - Recent clinical trials used ceftriaxone and azithromycin as the standard of care. Do not have ceftriaxone monotherapy clinical PK/PD data and no outcome data.
    - FDA funded *in vitro* (hollow fiber model) PK/PD studies for *N. gonorrhoeae* vs ceftriaxone alone and azithromycin alone. These data are the most robust assessment for these two drugs.
- New Breakpoint Issue – FDA Azithromycin Breakpoints
  - On 16 January 2025, FDA posted breakpoints for azithromycin via the STIC website

FDA and CLSI Azithromycin Breakpoints for *Neisseria gonorrhoeae*

	Minimum Inhibitory Concentrations (mcg/mL)		
	S	I	R
FDA	-	≤ 1	≥ 2
CLSI	≤ 1	-	-

Abbreviations: S, susceptible; I, intermediate; R, resistant;

  - Rationale: "FDA agrees with CLSI's assessment of surveillance data that < 1 mcg/mL is the end of the natural wild-type distribution and there are few isolates with MICs > 1 mcg/mL. FDA is not aware of pharmacokinetic/pharmacodynamic data that would inform breakpoints. With respect to clinical outcome data, it is FDA's assessment that published reports are not supportive of establishing a susceptible breakpoint. In a series from public sexually transmitted disease clinics in the U.S. published in the early 1990s, a high treatment success rate was reported for monotherapy with a single oral 2-gram azithromycin dose. However, in a clinical trial evaluating a single 2 gram oral dose of azithromycin with extended release formulation, failures of eradication were reported for subjects with isolates with MIC as low as 0.5 mcg/mL."
- Breakpoint Working Group Discussion and Recommendation
  - What about other cephalosporins in CLSI M100 for GC?
    - Cefoxitin, cefepime, cefotaxime, cefotetan, ceftizoxime (address later)
  - What PK-PD data were used to revise the cephalosporin dose recommendation?
    - Will review with CDC
  - What about ertapenem?

SC DISCUSSION (MAIN POINTS)

- The FDA may want PK/PD data to be reviewed.
- What about SDD?
  - There were only two cases, so too limited data.
  - Ertapenem has also been used for recalcitrant cases.
- CDC must have known the PK/PD data because they went higher on the dose for ceftriaxone.

CEFIDEROCOL DISK BREAKPOINTS FOR *STENOTROPHOMONAS MALTOPHILIA*

- CLSI *S. maltophilia* cefiderocol breakpoint history

	MIC breakpoints, µg/mL			Disk breakpoints, mm		
	S	I	R	S	I	R
June 2018 meeting (Inv.)	≤ 4	8	≥ 16	---	---	---

	MIC breakpoints, µg/mL			Disk breakpoints, mm		
	S	I	R	S	I	R
January 2019 meeting (Inv.)	≤ 4	8	≥ 16	≥ 15	12-14	≤ 11

Disk correlate data: 82 isolates (80 with MICs ≤ 1 µg/mL)

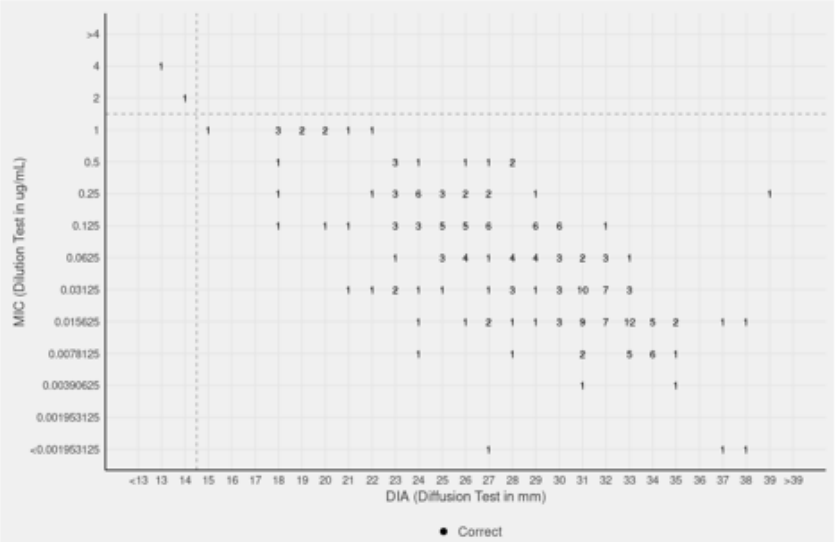
	MIC breakpoints, µg/mL			Disk breakpoints, mm		
	S	I	R	S	I	R
January 2021 meeting	≤ 1*	---	---	≥ 15*	---	---

Disk correlate data: 206 isolates (204 with MICs ≤ 1 µg/mL)

*Breakpoints are based on PK/PD properties, MIC distributions, and limited clinical data.

FDA did not recognize the CLSI breakpoints (and did not set their own)

- Using the disk correlates selected by CLSI, all isolates in the Shionogi data set were classified correctly



Current CLSI breakpoints

	S	I	R
MIC	≤ 1	---	---
Disk	≥ 15	---	---

No very major errors
No major errors

Note: **All but 2 of the 206 isolates had MICs in the susceptible range** (making it challenging to definitively say where the cutoff should be)

- EUCAST approach
  - Shared by Christian Giske

- MIC ≤0.5 mg/L (ZD ≥28 mm) lacks resistance mechanisms
- MICs 1-2 mg/L may have acquired resistance which may impair clinical response
- MIC >2 mg/L (ZD <22 mg) likely to be resistant

Stenotrophomonas maltophilia

Expert Rules and Expected Phenotypes

EUCAST Clinical Breakpoint Tables v. 15.0, valid from 2025-01-01

For abbreviations and explanations of breakpoints, see the Notes sheet

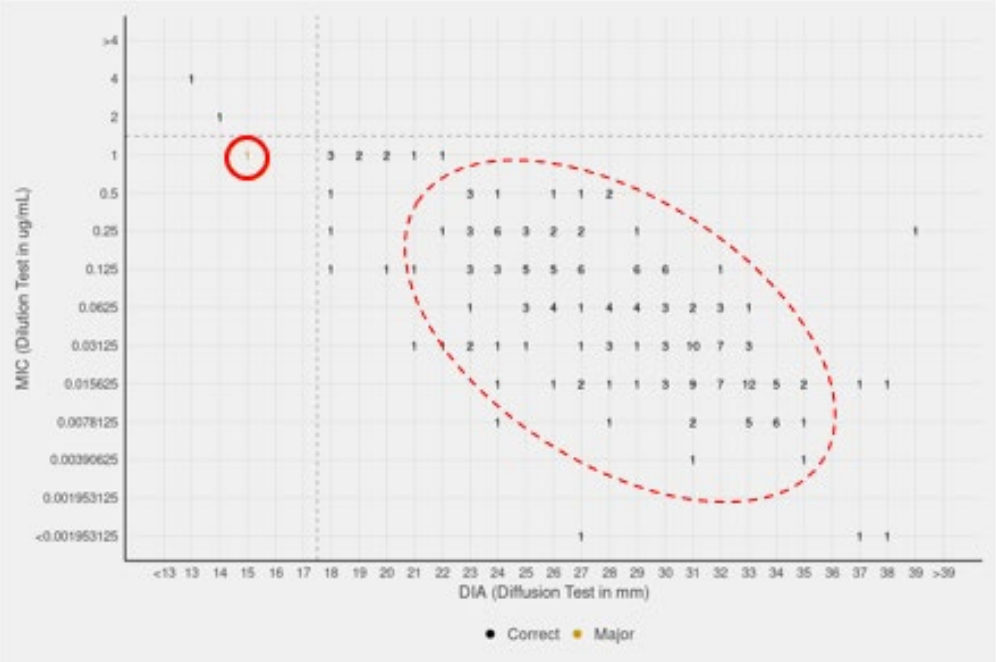
For further information, see EUCAST Guidance Document for *S. maltophilia*.

MIC determination (broth microdilution according to ISO standard 20776-1)
Medium: Cation-adjusted Mueller-Hinton broth (for cefiderocol, see https://www.eucast.org/eucastguidancedocuments/)
Inoculum: $5x10^5$ CFU/mL
Incubation: Sealed panels, air, 35±1°C, 18±2h
Reading: For trimethoprim-sulfamethoxazole, the MIC should be read at the lowest concentration that inhibits approximately 80% of growth as compared with the growth control well. See "EUCAST Reading Guide for broth microdilution" for further information.
Quality control: *Escherichia coli* ATCC 25922

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1°C, 18±2h
Reading: Read zone edges from the back of the plate against a dark background illuminated with reflected light (see below for specific instructions). See "EUCAST Reading Guide for disk diffusion" for further information.
Quality control: *Escherichia coli* ATCC 25922

Cephalosporins	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ceftazidime	-	-			-	-		1. Broth microdilution MIC determination must be performed in iron-depleted Mueller-Hinton broth and specific reading instructions must be followed. For testing conditions and reading instructions, see https://www.eucast.org/eucastguidancedocuments/.
Cefepime	-	-			-	-		
Cefiderocol[1]	Note[2]	Note[2]		30	Note[A]	Note[A]		2/A. The *in vitro* activity of cefiderocol against *Stenotrophomonas maltophilia* is comparable to the activity of the agent against *Enterobacterales* and there is also animal data to suggest efficacy. However, there is insufficient clinical data to determine a clinical breakpoint. Isolates with MIC values ≤0.5 mg/L (zone diameter ≥28 mm) are mostly devoid of resistance mechanisms. Isolates with MICs 1-2 mg/L have acquired resistance mechanisms which may result in impaired clinical response. Isolates with MIC values >2 mg/L (zone diameter <22 mm) will likely be resistant.

- Request from FDA CDER for CLSI to review disk correlates (10/3/2024)
  - "We are reviewing cefiderocol breakpoints against *S. maltophilia*. CLSI set a susceptible breakpoint at an MIC of ≤ 1 µg/mL with a corresponding zone diameter breakpoint of ≥ 15 mm. Based on our assessment, however, a zone diameter breakpoint of ≥ 18 mm seems to correspond more appropriately to the susceptible MIC breakpoint of ≤ 1 µg/mL with acceptable error rates. Please let us know if you have any comments or concerns about our findings. We would appreciate if your team could provide a response by October 24, 2024."
  - Data was reviewed, including email discussions with the Breakpoints Working Group and the Cefiderocol AHWG (under Methods Working Group). Comments were synthesized and provided to FDA before their requested deadline.
- FDA's proposal to shift the disk breakpoint from ≥ 15 mm to ≥ 18 mm would introduce a single major error
  - A cutoff of ≥ 18 mm would abut a group of susceptible isolates, including a few with MICs < 1 µg/mL
  - Particularly given the variability when this drug is tested (including variability based on media brand), leaving no buffer there could result in occasional false non-susceptible calls – that said, the zone sizes for most susceptible isolates are larger



- Error rates would meet CLSI M23 criteria at cutoffs of ≥ 15 mm, ≥ 16 mm, ≥ 17 mm, or ≥ 18 mm

≥ 14 S, ≤ 13 NS

	n	VME, %	ME, %
≥ NS + 1	1	0	NA
NS + S	11	1 (9.1)	0
≤ S - 1	194	NA	0

But the single VME represents 1 out of 2 (50%) R isolates.

≥ 15 S, ≤ 14 NS (current CLSI)

	n	VME, %	ME, %
≥ NS + 1	1	0	NA
NS + S	11	0	0
≤ S - 1	194	NA	0

≥ 16 S, ≤ 15 NS

	n	VME, %	ME, %
≥ NS + 1	1	0	NA
NS + S	11	0	1 (9.1)
≤ S - 1	194	NA	0

≥ 17 S, ≤ 16 NS

	n	VME, %	ME, %
≥ NS + 1	1	0	NA
NS + S	11	0	1 (9.1)
≤ S - 1	194	NA	0

≥ 18 S, ≤ 17 NS (FDA proposal)

	n	VME, %	ME, %
≥ NS + 1	1	0	NA
NS + S	11	0	1 (9.1)
≤ S - 1	194	NA	0

≥ 19 S, ≤ 18 NS

	n	VME, %	ME, %
≥ NS + 1	1	0	NA
NS + S	11	0	4 (36.4)
≤ S - 1	194	NA	3 (1.5)

Too many major errors

CLSI M23Ed6, 2023.

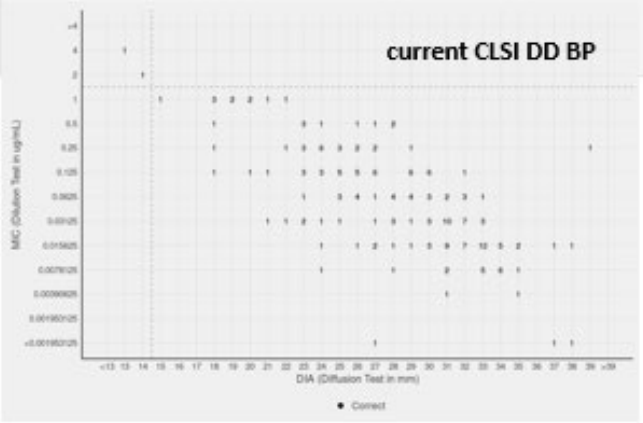
	VME, %	ME, %
≥ NS + 1	<2	NA
NS + S	<10	<10
≤ S - 1	NA	<5

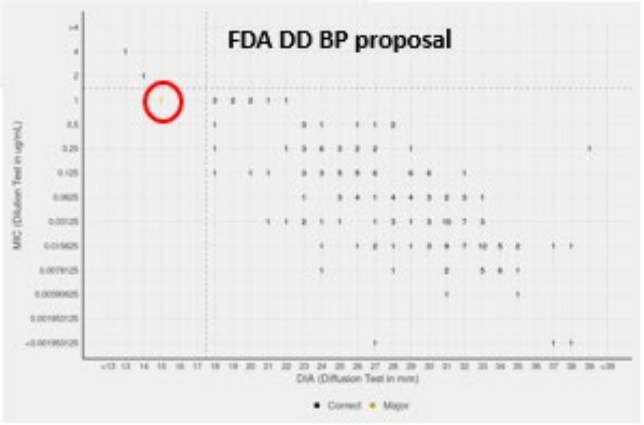
- dBETs
  - In a bootstrap analysis of the error-rate bounded analysis, dBETs selected the current CLSI cutoff of ≥ 15 mm 54.88% of the time
  - The FDA's proposal of ≥ 18 mm was the second most frequently selected (40.18% of the time).
  - Although dBETS selected the CLSI cutoff of ≥ 15 mm as optimal, it acknowledges uncertainty

Bootstrap samples = 12000

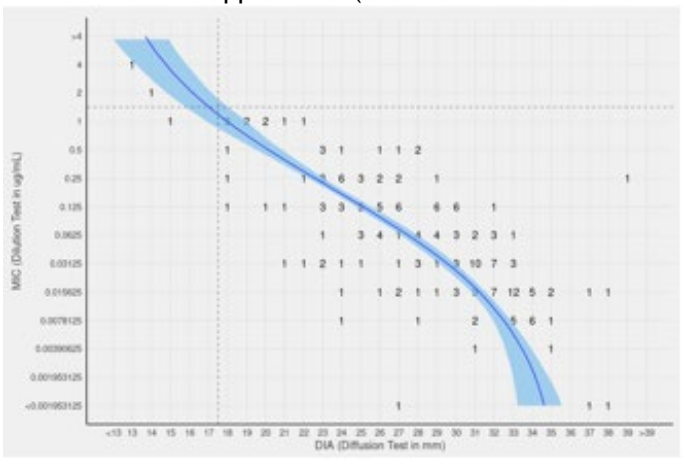
-------DIA Breakpoints by Confidence--------

DIABrkpt	Percent	Cumulative
15	54.88	54.88
18	40.18	95.07

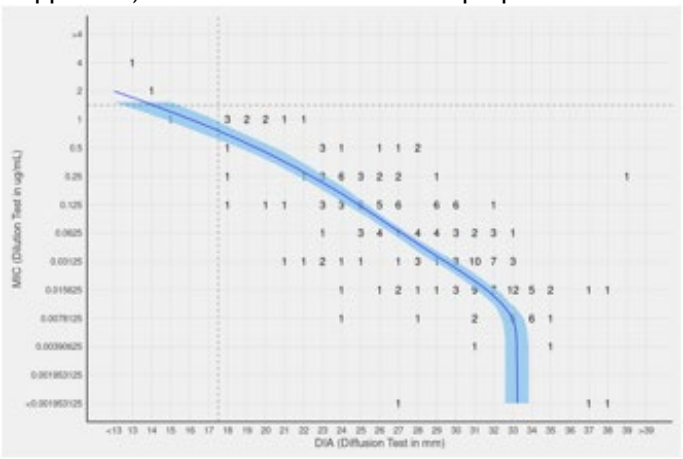




- Model-based approaches (rather than the error-bound approach) in dBETS select the FDA's proposed cutoff of ≥ 18 mm as the optimal fit for the data



Four-parameter logistic model



Nonparametric spline model

- Variability is observed (including media manufacturer-related differences) when testing cefiderocol by disk diffusion for *S. maltophilia*

- Laboratory Specialists, Inc. has *S. maltophilia* cefiderocol verification panel
- Variability is observed in zone sizes across media brands
- For some isolates, there is substantial variability in zone sizes even when using a single media brand
- Reading cefiderocol disk zones is generally straightforward for *S. maltophilia*
- There is a single isolate in this set (#1) for which a small number of inner colonies appear – most zones were ≥ 18 mm, but 3 out of 60 measurements were ≤ 17 mm (2 at 17 mm and 1 at 16 mm)

Zones of Inhibition (mm)

		MHA	BD																	
Isolate	MIC	Disk	BD					Hardy									Oxoid				
S. maltophilia #31	0.12		33	30	32	32	34	30	30	29	31	32	32	30	31	30	33	33	32	34	33	35
S. maltophilia #32	0.25		32	32	32	33	34	30	31	33	33	32	31	31	31	32	32	32	34	34	34	35
S. maltophilia #33	0.12		32	30	30	31	32	29	29	29	30	30	30	30	30	29	31	31	31	32	32	33
S. maltophilia #34	>32		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
S. maltophilia #35	1		27	26	28	28	28	22	23	22	22	21	26	27	28	25	27	28	30	30	29	29

Zones of Inhibition (mm)

		MHA	Hardy																	
Isolate	MIC	Disk	BD					Hardy									Oxoid				
S. maltophilia #1	0.12		29	29	28	30	30	28	29	28	29	18	29	28	27	29	30	31	31	31	30	32
S. maltophilia #2	0.25		22	24	22	24	25	26	26	27	27	26	21	24	24	23	24	24	26	25	25	27
S. maltophilia #3	0.12		27	27	26	28	28	27	27	28	28	27	27	26	26	26	27	27	29	29	28	30
S. maltophilia #4	>32		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
S. maltophilia #5	1		20	19	19	19	18	20	21	21	20	19	19	19	17	20	19	21	21	22	21	20

Zones of Inhibition (mm)

		MHA	Remel																	
Isolate	MIC	Disk	BD					Hardy									Oxoid				
S. maltophilia #1	0.12		28	29	28	28	30	28	28	27	28	17	29	29	27	27	28	30	30	29	29	32
S. maltophilia #2	0.25		24	21	26	24	27	26	28	22	25	26	24	21	25	23	26	25	24	27	25	29
S. maltophilia #3	0.12		28	27	27	27	28	25	28	27	26	27	27	26	26	27	28	29	29	29	28	30
S. maltophilia #4	>32		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
S. maltophilia #5	1		19	18	17	18	18	20	20	18	19	18	18	18	16	18	18	20	21	20	20	21

- The EUCAST Development Laboratory cefiderocol disk correlate data also include few isolates with MICs > 1 µg/mL
  - 80 isolates tested on MH agar from 2 manufacturers (BBL and Oxoid) and in ID-CAMHB prepared from CAMHB from 2 manufacturers (BBL and Difco) = 320 correlates
  - No isolates with MICs > 2 µg/mL
  - 3 isolates with MICs 2 mg/L in ID-CAMHB BBL (but 0.5 mg/L in ID-CAMHB Difco) and zones between 24 and 31 mm

- Zone diameters not difficult to read, but MIC endpoints can be difficult to read
- Data courtesy of Erika Matuschek

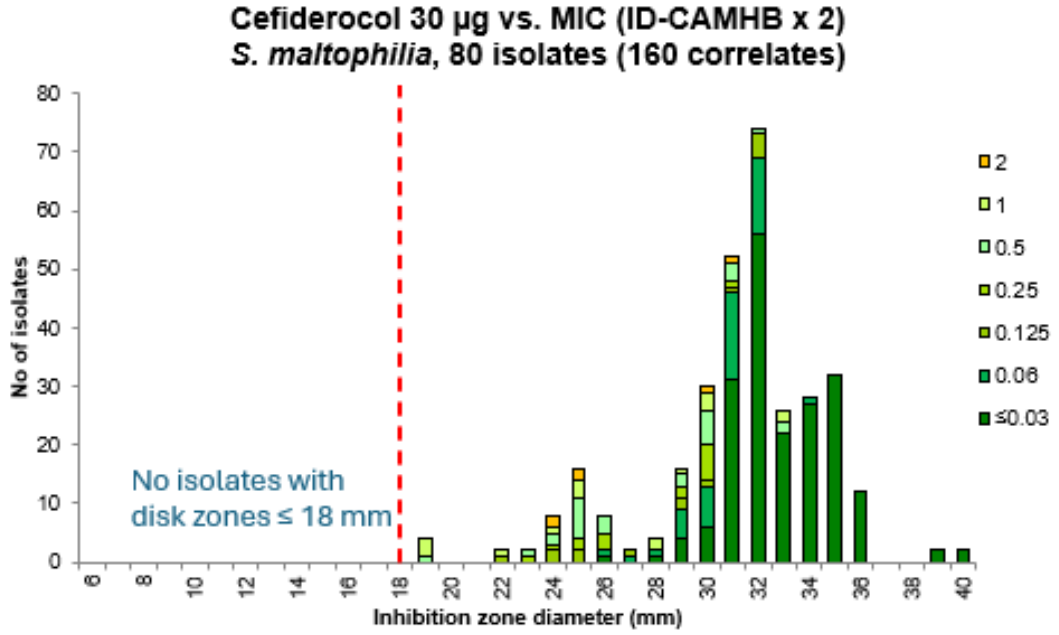
***S. maltophilia* and cefiderocol**

80 isolates tested on MH agar from 2 manufacturers and in ID-CAMHB from 2 manufacturers (320 correlates)

MIC ID-CAMHB (mg/L)	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
≥64																																			
32																																			
16																																			
8																																			
4																																			
2																			2	2					1	1									
1														3		1			1	3			2	1	3			2							
0.5														1				1	2	7	3			2	6	3	1	2							
0.25																		1	1	2	3			2	6	1									
0.125																	1		2	2		1		2	1	1	4								
0.06																					1	1	1	5	7	15	13		1						
≤0.03																					1		1	4	6	31	56	22	27	32	12			2	2

Inhibition zone diameter (mm)

- Isolates with MICs of 2 µg/mL had zone diameters from 24-31 mm, precluding setting a disk correlate that would separate them from isolates with MICs ≤ 1 µg/mL



- Summary of email discussion

- Ideally, would have more resistant isolates to assess the performance in a disk correlate dataset, but could not find such data (including in an updated literature search)
- Members of the Breakpoints Working Group and the Cefiderocol AHWG generally favored harmonization between CLSI and FDA for the *S. maltophilia* disk correlates
- However, given the variability in disk zones when cefiderocol is tested on different brands of media, the prevailing opinion was to propose setting the cutoff at ≥ 17 mm susceptible (rather than ≥ 18 mm susceptible) to provide more of a buffer against overcalling resistance, especially given that there are relatively few available treatment options for infections caused by this organism

- FDA decision
  - FDA STIC website updated 11/12/2024
  - "FDA concludes that available PK/PD data coupled with the MIC distribution data and the ECV value of 1 mcg/mL support the recognition of the current CLSI MIC breakpoint for cefiderocol against *S. maltophilia*, ie, a susceptible only breakpoint at an MIC of ≤ 1 mcg/mL. As for a susceptible disk diffusion breakpoint (DD BP), FDA concluded that the diameter of a zone of inhibition of ≥ 17 mm provides a more optimal fit using a model-based approach as compared to the DD BP of ≥ 15 mm (the current CLSI disk diffusion susceptible breakpoint). In addition, given variability in test media, the susceptible DD BP of ≥ 17 mm would mitigate the risk of overcalling susceptibility as compared to ≥ 15 mm, while providing acceptable error rates."
- Proposal to the Breakpoints Working Group: Revise the *S. maltophilia* cefiderocol disk diffusion breakpoint to ≥ 17 mm susceptible, which will harmonize with the recently established FDA STIC
- Breakpoint Working Group Discussion and Recommendation
  - EUCAST perspective shared no breakpoint but instead has a "Note"
  - One member in favor of original FDA proposal (≥18 mm) to be more conservative
  - More recent papers with MICs for *S. maltophilia* with higher than 1 mg/L (current MIC breakpoint)
  - Motion to accept proposed change to cefiderocol disk diffusion breakpoint for *S. maltophilia* from ≥15 mm (S) to ≥17 mm (S). WG Vote: 9-1-0-3 (no government representation).

SC DISCUSSION (MAIN POINTS)

- Consensus is to revise the *S. maltophilia* susceptible breakpoint to ≥ 17 mm for disk diffusion.
- The *Stenotrophomonas* isolates with MICs of 2 µg/mL in the EUCAST study tested at 0.5 µg/mL using different media, so there were issues with MIC testing.
- Think about how QC data can help account for the disk diffusion.

A motion to accept the disk (30 µg) diffusion cefiderocol susceptible breakpoint (≥17 mm) for *Stenotrophomonas maltophilia* was made and seconded. Vote: 13 for, 0 against, 0 abstain, 1 absent (Pass)

TRIMETHOPRIM-SULFAMETHOXAZOLE (SXT) BETA-STREPTOCOCCI AD HOC WORKING GROUP REPORT

- AHWG Charge
  - Reviewing the available data to make an informational presentation for discussion at the January 2025 CLSI Breakpoint Working Group and AST Subcommittee Meeting
  - Craft a voting proposal for the June 2025 AST Subcommittee Meetings for publication in the subsequent edition of CLSI M100
- Microbiology

- Large colony forming pyogenic strains
- Species from the pyogenic or beta-hemolytic group are further characterized by the presence of Lancefield antigens, which do not always correlate with the proper streptococcal species designations

Lancefield Antigen Group	Organisms
Group A	*S. pyogenes*
Group B	*S. agalactiae*
Group C	*S. dysgalactiae* subsp. *equisimilis* *S. dysgalactiae* subsp. *dysgalactiae* (alpha-hemolytic) *S. equi* subsp. *equi* *S. equi* subsp. *zooepidemicus*
Group G	*S. dysgalactiae* subsp. *equisimilis* *S. canis*

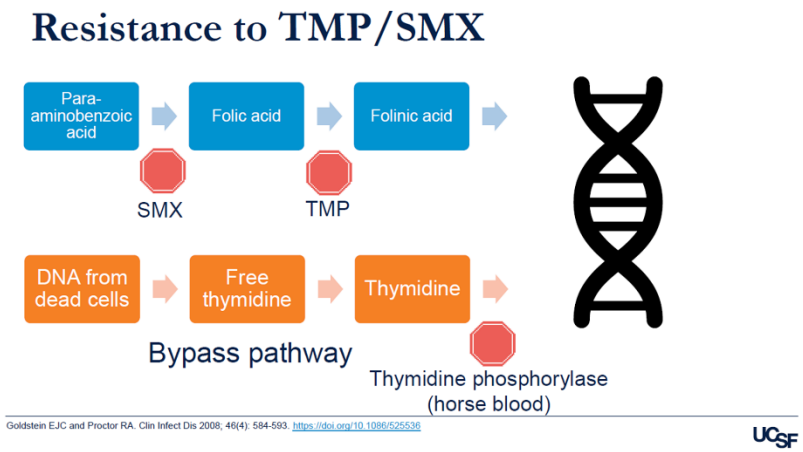
- Antimicrobial Susceptibility Testing Methods
  - SXT disk content the same for CLSI and EUCAST breakpoints: 1.25/23.75 µg

	Disk Diffusion	Broth Microdilution	Agar Dilution	SXT Breakpoints?
CLSI	MHA with **5% sheep blood** 35°C ± 2°C 5% CO_2; 20–24 hours	**CAMHB with lysed horse blood** (2.5% to 5% v/v) 35°C ± 2°C ambient air; 20–24 hours	**MHA with sheep blood** (5% v/v) 35°C ± 2°C ambient air; 20–24 hours (CO_2 if necessary, for growth with agar dilution)	**No**
EUCAST	Mueller-Hinton agar + **5% defibrinated horse blood** and 20 mg/L β-NAD (MH-F) Sealed panels, air, 35±1ºC, 18±2h (for glycopeptides 24h)	**Cation-adjusted Mueller-Hinton broth + 5% lysed horse blood** and 20 mg/L β-NAD (MH-F broth) 5% CO2, 35±1ºC, 18±2h	Guidance not available	**Yes**

- Beta-hemolytic Streptococci and SXT
  - Early studies demonstrated beta-hemolytic streptococci had variable rates of resistance to SXT
    - Method dependent

- Perpetuated the thought that beta-hemolytic streptococci had high levels of resistance to SXT (or even thought to be intrinsically resistant)
- Fun fact: SXT has been incorporated into selective media to isolate beta-hemolytic streptococci from throat swabs

- What is the difference between sheep and horse blood?
  - Lysed horse blood contains thymidine phosphorylase, which neutralizes thymidine
  - Thymidine phosphorylase converts thymidine to thymine and hence overcomes the inhibition of folate metabolism that occurs in the presence of thymidine
  - Can no longer serve as an exogenous source of thymidine
  - No other mammalian blood carries thymidine phosphorylase
- Resistance



- Regulating Thymidine Content in MHA
  - High thymidine content (>0.03 mg/l) in agar provides an exogenous substrate which can be used by an organism to maintain folate metabolism and hence appear resistant to SXT
  - M06-A2 Evaluating production lots of dehydrated MHA
    - 2006: started to regulate thymidine content
    - No longer necessary to add lysed horse blood to the medium
    - Document is withdrawn and was replaced with ISO 16782 (MHA and broth for AST)
- Studies
  - Bowen et al JCM 2012 Study
    - SXT Etest results on different media
    - Demonstrated elevated MICs on MHA with sheep blood relative to MHF and MHA (without any blood added)
  - Nottebrock H and Then R. Thymidine Concentrations in Serum and Urine of Different Animal Species and Man. 1977

- Imöhl M, van der Linden M (2015) Antimicrobial Susceptibility of Invasive *Streptococcus pyogenes* Isolates in Germany during 2003-2013. PLoS ONE 10(9): e0137313.
  - Study from Germany
  - *S. pyogenes* 2003-2013
  - Applied CLSI BMD method
  - Interpreted applying EUCAST breakpoints
  - High rates of susceptibility (>98%)
- Cho C, et al. In vitro activity of clindamycin, doxycycline, and trimethoprim/sulfamethoxazole against clinical isolates of ß-hemolytic *Streptococcus* spp. via BD Phoenix and broth microdilution. Antimicrob Steward Healthc Epidemiol. 2023 Dec 15;3(1):e238.
  - Comparison of beta-hemolytic streptococci SXT susceptibility by BMD (5% LHB-MHB) and BD Phoenix SMIC-101 *Streptococcus* panel
  - Low concordance (high rate of major errors) when testing beta-hemolytic streptococci SXT susceptibility on BD Phoenix

- EUCAST Disk Correlate Data on MHF available
- How to QC Thymidine Content?

CLSI - M02-Ed14

3.1.1.2 Effects of Thymidine or Thymine

MHA containing excessive amounts of thymidine or thymine can reverse the inhibitory effect of sulfonamides and trimethoprim, thus yielding smaller and less distinct zones, or no zone at all, which may result in false-resistance reports. The laboratory should use MHA that is as low in thymidine content as possible. If problems with sulfonamide and trimethoprim QC occur, it might be necessary to check the MHA. To evaluate an MHA lot, *E. faecalis* ATCC® 29212 or, alternatively, *E. faecalis* ATCC® 33186 may be tested with trimethoprim-sulfamethoxazole disks. Satisfactory media provide essentially clear, distinct zones of inhibition that are ≥ 20 mm. Unsatisfactory media produce no zone of inhibition, growth within the inhibition zone, or a zone of inhibition < 20 mm.

CLSI - M07-Ed12

A3.4 *Haemophilus* Test Medium Broth

In its broth form, *Haemophilus* test medium (HTM) consists of:

- MHB
- 15 µg/mL NAD
- 15 µg/mL bovine or porcine hematin
- 5 g/L yeast extract
- 0.2 IU/mL thymidine phosphorylase (if sulfonamides or trimethoprim are to be tested)

- Thoughts
  - If approve setting SXT breakpoints for beta-hemolytic streptococci, recommend reference BMD MIC testing if performed
  - Maybe later - Perform a method evaluation study
    - MHA + 5% sheep and MHF for agar based methods compared to reference BMD
      - Disk and gradient diffusion
      - Assess disk correlates
    - Focus on *S. pyogenes* or evaluate all beta-hemolytic streptococci?

- Should other agents be evaluated at the same time?
    - Confusing to use MHA + 5% sheep's blood for all drugs but MHF for SXT only (prone to error)
- Table 1 Test and Report Recommendation
    - Once methods are determined, clinical indications, and approach, will need to address recommendations for testing and reporting SXT for beta-hemolytic streptococci

- PK/PD Analysis
    - PK/PD targets for beta-hemolytic streptococci remain undefined
- Clinical Data
    - Clinical data pertaining to SXT vs beta-hemolytic streptococci
        - Is SXT effective against infections caused by beta-hemolytic streptococci?
        - Most published studies (predominantly uSSTI and cSSTI) do not answer this question (wrong patient population, wrong comparators)
        - Very limited data from RCTs comparing SXT to standard of care for infections shown to be or likely to be caused by beta-hemolytic streptococci
        - With those caveats the preponderance of available data suggest efficacy with proper dosing
    - Studies
        - Bowen AC, et al. Short-course oral co-trimoxazole versus intramuscular benzathine benzylpenicillin for impetigo in a highly endemic region: an open-label, randomised, controlled, non-inferiority trial. Lancet. 2014 Dec 13;384(9960):2132-40.
            - *S. pyogenes* isolated from 90% baseline
            - 74% had both *S. pyogenes* and *S. aureus*
            - 5 (1%) of patients had SXT resistant *S. pyogenes* isolates
            - All penicillin susceptible
        - Trickett, J Infect Dis. 1973 Nov:128:Suppl:693-5
            - 96 patients enrolled, 87 evaluable (excluded – lost to follow-up, failure to take study medication per protocol, negative group A streptococci serology)
            - Conversion to negative culture
                - SXT – 43/44 patients converted to negative culture
                    - 26 sterile day 2
                    - 13 more day 4
                    - 4 more day 10
                - Penicillin G – 42/43 patients converted to negative culture
                - 41/42 negative culture day 2
            - Recurrence during study period- considered relapse not reinfection
            - SXT – 12/44 patients had recurrences
            - Penicillin G – 4/43 patients had recurrences
        - Hedin Cochrane Database Syst Rev. 2023 Nov 13; 11(11):CD004406.
            - Nothing new on SXT since 1973
    - Clinical Data Summary
        - Clinical data are incomplete
        - Available data for uSSTI and cSSTI suggest efficacy

- Available data for group A streptococci pharyngitis raise a question regarding efficacy vs comparator (Penicillin G), but
  - Dosing of SXT in that study may not be adequate (?weight of patients)
  - Given the small sample size, 95% CI crosses 1

- SXT Beta-Hemolytic Streptococci AHWG Discussion and Recommendations
  - Reasonable to consider SXT breakpoints for *S. pyogenes* at least for uncomplicated SSTI
  - Data for other clinical indications and beta-hemolytic streptococci species is very limited
    - Most non-purulent cellulitis is treated without cultures, so perhaps include all beta-hemolytic streptococci?
    - uUTI?
    - Limit reporting to specific specimen types (ie, do not report for blood)?
  - Limited and extrapolated PK/PD data suggest that a PTA for stasis based on arbitrary $fAUC_{0\text{-}24}$ /MIC target of 25 up to an MIC of 0.5 mg/L for 5 mg/kg/day or 1 DS tab twice daily
  - EUCAST: ECV of ≤0.5 µg/ml for beta-hemolytic streptococci
  - AST should be limited to reference BMD MIC testing
    - Problem: Limited availability
    - Future studies evaluating alternative media (MHF) and disk correlates could be considered for all agents
- Breakpoint Working Group Discussion and Recommendation
  - Assess which isolates are appropriate for testing (Trish Simner)
    - Limit to specific specimen types?
  - Clinical context (Howard Gold): for SSTI, SXT and ß-lactam is often used to cover for MRSA and beta-hemolytic streptococci
  - Consider utility of breakpoints
    - Patient-specific clinical use (test and report)
    - Surveillance/antibiogram (test and do no report)
  - Setting breakpoints for beta-hemolytic streptococci vs group A streptococci only?
  - Placement in Table 1
  - EUCAST: working to lower breakpoint down to ECV

SC DISCUSSION (MAIN POINTS)

- When would laboratories perform testing? What about vaginal colonization? Would it be appropriate to test?
- Majority would like to see data for all beta-hemolytic streptococci.
- In the Australia study, there were a lot of co-infections with *S. aureus*. *S. aureus* in Australia have high penicillin susceptibly.
- NEJM 2015 clindamycin vs SXT for skin and soft tissue infection data does exist.
  - The AHWG did read this paper.
- Do not want people using SXT for bacteremia or severe infections. Consider if laboratories test behind the scenes and then the data is kept on an antibiogram.
  - It is generally not realistic for laboratories to test a drug if it will not be reported on each patient.
  - Laboratories do not know if an infection is severe. Consider placing this drug in Tier 4 in Tables 1. State that if you do need to test, then recommend it is done by BMD.
  - Some not in favor of Tier 4.
- Do commercial methods work?

- There are some indicators that the commercial methods do not work.
- In the drug label, it says SXT will not treat group A streptococcal pharyngitis.
- Clinical laboratories are not at a point to routinely test. The goal now is to get the message out that these organisms are not intrinsically resistant to SXT.
- There were some treatment failures in UTI going to bacteremia. Is there a worry about tetrahydrofolates being introduced from clinical specimens?
  - Counter point, if someone would have gotten SXT plus cephalexin for SSTI that also would not have saved them from a deep seated infection anyways. So, an SXT breakpoint would not change the outcome.
- Stewardship teams have been moving to using SXT for a few years. Academic medical centers are using SXT for uncomplicated SSTI and it has been working.
- Phase III randomized clinical trial in NEJM trial says SXT works. Physicians have been using SXT in outpatient settings. Group B cellulitis in diabetics is routinely treated with SXT plus amoxicillin-clavulanate then the patient gets a rash from amoxicillin-clavulanate. Stewardship pushes providers to drop amoxicillin-clavulanate to prevent the rash, but then providers push back because they are concerned that the laboratory cannot give them an SXT susceptibility result.
- Sometimes too much onus is put on laboratories and not enough responsibility on physicians.
- There was a concern there is not enough data to make a decision.
- UCLA has reference method MIC data.
- Consider looking at SXT and *S. aureus* data too since it is going to be in all the same studies already being reviewed.
- If all the isolates look like wild-type and have clinical outcome data in SSTI, then can infer activity of the drug.
- Reminder that without an FDA recognized breakpoint, commercial manufacturers cannot put it on their panels.
- If anyone has SXT data on a panel even if not reported, please share the data.
- Action Item: SXT Beta-Hemolytic Streptococci AHWG assemble the data for a SXT and beta-hemolytic streptococci breakpoint. Then the Breakpoints Working Group should review the data and determine if there is enough to set a breakpoint or an ECV.

PENICILLIN AND GROUP B STREPTOCOCCI BREAKPOINTS

- Penicillin-resistant group B streptococci (GBS) was included on the 2024 WHO Bacterial Priority Pathogens List as a medium priority
- Discussions with WHO
  - The WHO Advisory Group was contacted to discuss the reason behind this change in the priority pathogen list update
  - WHO answered that this change was made to encourage the development of a vaccine against GBS because:
    - GBS colonizes 18% of pregnant women worldwide with 6% of neonates having invasive disease like meningitis, sepsis and pneumonia.
    - Intrapartum antibiotic prophylaxis only addresses early-onset of GBS infection. But is not protective against late-onset diseases.
    - Side effects, development of resistance and dysbiosis in neonates, is a matter of concern.
    - A vaccine would prevent GBS infections due to the ability of maternal immunoglobulin G to cross the placenta and provide immunity to the fetus.
  - To date, WHO does not provide any recommendations about AST in GBS.
- Issues with AST for GBS that CLSI should be aware of:
  - Most clinical microbiology laboratories in the US and abroad are not performing AST for GBS. Many institutions perform rapid molecular methods (eg, PCR), but are not culturing, so they do not get colonies for AST.
  - Clinical microbiology laboratories performing AST are not testing for β-lactams (eg, penicillin/ampicillin), as they are assuming that GBS are susceptible.

- CLSI M100 does not have resistant category for ß-lactams in beta-hemolytic group tables (1H-1). By contrast EUCAST established a resistant category for streptococci A, B, C and G.
- Recent reports from proficiency testing surveys are showing limitations with different methodologies to detect penicillin resistance in GBS. From disk-diffusion to commercial broth microdilution devices. Also, participants included results in categories not established by CLSI like intermediate and resistant.
- There are several publications from countries in Africa, Central America, Asia, etc, reporting penicillin resistance in GBS. However, they are using mixed methods and are not taking into account limitations. (They reference CLSI M100).

- GBS Mechanisms of Resistance
  - PBP mutations (substitutions) affecting the binding capacity of ß-lactams.
  - Several mutations have been identified in PBP1a, PBP2a, PBP2b, PBP2x.
  - However, the most important mutations are V405A and Q557E in the PBP2x transpeptidase.
- Reports of penicillin resistant GBS
  - J Antimicrob Chemother. 2024 Nov 15:dkae419
  - J Antimicrob Chemother. 2015;70(6):1601-3
  - Crit Rev Microbiol. 2020 May;46(3):253-269
  - Pathogens. 2022 Mar 29;11(4):415
- CLSI M100 Table 1H-1

Table 1H-1. *Streptococcus* spp. β-Hemolytic Group

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Clindamycin[a,b]			
Erythromycin[a,b,c]			
Penicillin[d] or ampicillin[d]		Cefotaxime or ceftriaxone	Cefepime
			Ceftaroline
	Tetracycline[e]		
		Vancomycin	
			Linezolid
			Tedizolid[f]
			Daptomycin[g,h]
			Levofloxacin
			Dalbavancin[h,i]
			Oritavancin[h]
			Telavancin[h]

. Penicillin and ampicillin are drugs of choice for treating β-hemolytic streptococcal infections. Susceptibility testing of penicillins and other β-lactams approved by the FDA for treatment of β-hemolytic streptococcal infections does not need to be performed routinely, because nonsusceptible isolates (ie, penicillin MICs > 0.12 and ampicillin MICs > 0.25 µg/mL) are extremely rare in any β-hemolytic streptococci and have not been reported for *S. pyogenes*. If testing is performed, any β-hemolytic streptococcal isolate found to be nonsusceptible should be re-identified, retested, and if confirmed, submitted to a public health laboratory (see Appendix A for additional instructions).

- CLSI M100 Table 2H-1 compared with EUCAST

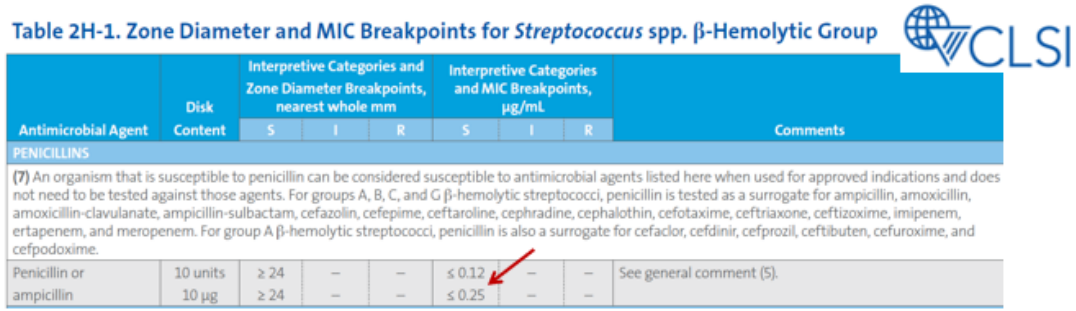


Table 2H-1. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. β-Hemolytic Group

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm S	I	R	Interpretive Categories and MIC Breakpoints, µg/mL S	I	R	Comments
PENICILLINS								
(7) An organism that is susceptible to penicillin can be considered susceptible to antimicrobial agents listed here when used for approved indications and does not need to be tested against those agents. For groups A, B, C, and G β-hemolytic streptococci, penicillin is tested as a surrogate for ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefazolin, cefepime, ceftaroline, cephradine, cephalothin, cefotaxime, ceftriaxone, ceftizoxime, imipenem, ertapenem, and meropenem. For group A β-hemolytic streptococci, penicillin is also a surrogate for cefaclor, cefdinir, cefprozil, ceftibuten, cefuroxime, and cefpodoxime.								
Penicillin or ampicillin	10 units 10 µg	≥ 24 ≥ 24	– –	– –	≤ 0.12 ≤ 0.25	– –	– –	See general comment (5).

Streptococcus groups A, B, C and G

EUCAST

Penicillins[1]	MIC breakpoints (mg/L) S ≤	R >	ATU	Disk content (µg)	Zone diameter breakpoints (mm) S ≥	R <	ATU
Benzylpenicillin (indications other than meningitis)[2]	0.25	0.25		1 unit	18	18	
Benzylpenicillin (meningitis)[2], *S. agalactiae* (group B streptococci)	0.125	0.125		1 unit	19	19	
Ampicillin	Note[1]	Note[1]			Note[A]	Note[A]	
Ampicillin-sulbactam[3]	Note[1]	Note[1]			Note[A]	Note[A]	
Amoxicillin	Note[1]	Note[1]			Note[A]	Note[A]	

- CLSI M100 Appendix A

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
Streptococcus pneumoniae	Penicillins	Amoxicillin or penicillin (nonmeningitis) – R			X
	Cephems	Cephalosporin III/IV (nonmeningitis) – R			X
		Ceftaroline (nonmeningitis) – NS	X		
	Carbapenems	Any carbapenem – I, R, or NS		X	
	Glycopeptides	Vancomycin – NS	X		
	Fluoroquinolones	Any fluoroquinolone – I or R		X	
	Streptogramins	Quinupristin-dalfopristin – I or R		X	
	Ansamycins	Rifampin – I or R		X	
	Oxazolidinones	Linezolid – NS	X		
	Pleuromutilins	Lefamulin – NS	X		
Streptococcus, β-hemolytic group	Penicillins	Ampicillin or penicillin – NS	X		
	Cephems	Cephalosporin III/IV – NS Ceftaroline – NS	X		
	Carbapenems	Any carbapenem – NS	X		
	Glycopeptides	Vancomycin – NS	X		
	Lipoglycopeptides	Dalbavancin – NS Oritavancin – NS Telavancin – NS	X X X		
	Lipopeptides	Daptomycin – NS	X		
	Streptogramins	Quinupristin-dalfopristin (*S. pyogenes* only) – I or R		X	
	Oxazolidinones	Linezolid – NS Tedizolid – NS	X X		

- Detection of Penicillin Nonsusceptible *Streptococcus agalactiae* by Laboratories That Participate in the College of American Pathologist's Proficiency Testing Program
  - J. Clin Microbiol. 2023 Aug 23;61(8)
  - Proficiency Testing Survey in 2021 by the College of American Pathologists (CAP)
  - Laboratories were asked to identify the principal pathogen and perform antimicrobial susceptibility testing (AST).
  - The GBS isolate had been determined to have a penicillin MIC of 0.25 µg/mL by reference broth microdilution performed according to CLSI standards.
  - This isolate harbor a PBP2x mutation Q557E, which is known to be associated with decreased susceptibility to the ß-lactams.
  - 737 results
- Comments and Request to the Breakpoints Working Group
  - The inaccuracies of AST methods for ß-lactams in GBS is a matter of concern.
  - It is noteworthy that standard disk diffusion is not working either. The disk correlates may require revision.
  - According to our internal guidelines (CLSI M23), there is a defined mechanism of resistance, and several reports of penicillin resistance worldwide. That should promote the establishment of the intermediate and resistant breakpoints.
  - Should clarify what non-susceptible means for clinical laboratories and physicians.
  - Request to consider the creation of an AHWG to address AST with GBS and to analyze if a breakpoint revision is required at this point, but also to provide guidance to clinical microbiology and physicians about AST for GBS.
- Breakpoints Working Group Discussion and Recommendation

	○ Need clinical data ○ Some test invasive isolates (eg, IE) to think about dosing and "screen" for resistance mechanisms ○ What would be the charge if an AHWG formed? Areas to consider: ▪ Assess language in Table 1 ▪ Assess need to revision PCN breakpoint (lower S? add I and/or R?) ▪ GBS AST issues ○ EUCAST would aim to join the AHWG if formed ○ Motion to form an AHWG to address AST with GBS for the following items: ▪ (1) CLSI M100 Table 2H – address "routinely" comment ▪ (2) Evaluate breakpoint ▪ WG Vote: 9-0-0-4 (no government representation) **SC DISCUSSION (MAIN POINTS)** • There has not been an evidence of treatment failures. Remember that the CAP data indicates that laboratories would report an organism as susceptible, that does not mean the laboratories tested it. Laboratories might be reporting as susceptible without testing. • For treatment, high doses of penicillin and addition with a second drug are often used. The prophylaxis in the US has been incredibly successful. The CDC should be included in AHWG. • When penicillin breakpoints for group B streptococci were set long ago, the subcommittee knew that isolates might test non-susceptible, but the clinical failures were so vanishingly small that they decided to say testing is not needed and have a comment that resistance has not been detected. • Trish Simner and Romney Humphries might be able to do some preliminary evaluation of the disk correlates. • For the AHWG, it would be good for them to consider the disk diffusion correlates and to assess if there is a global signal. ○ Suggestion to check with the CDC first. • Suggestion to work with Japanese experts to see if there is any data of resistance. • Children can do poorly clinically for so many reasons that it could be hard to find a clinical signal. • The current comment in CLSI M100 is good to keep. • The penicillin dose is incredibly high and should clear even with the higher penicillin MICs. • CLSI needs to be judicious in deciding objectives for an AHWG. • Consider changing the comment to acknowledge that higher MICs exist, but perhaps there is no clinical signal. • Action item: Breakpoints Working Group gather a bit more data for June 2025 prior to forming an AHWG.
3.	**ADJOURNMENT** Dr. Mathers thanked the participants for their attention. The meeting was adjourned at 5:30 PM Eastern Standard (US) time.

2025 JANUARY AST MEETING SUMMARY MINUTES PLENARY 3: Tuesday, 28 January 7:30 AM – 12:00 PM Eastern Standard Time (US)	
#	**Description**
1.	**OPENING** Dr. Mathers opened the meeting at 7:30 AM Eastern Standard (US) time.

2. **METHODS WORKING GROUP (T. DINGLE AND K. JOHNSON)**

RIFABUTIN REFERENCE SUSCEPTIBILITY TESTING METHOD AGAINST *ACINETOBACTER BAUMANNII*

- January 2024 AST Subcommittee Decision

A motion to support the agar dilution + PIH method for rifabutin testing of *Acinetobacter baumannii* as the reference method for Tier 1 and Tier 2 QC studies with approval of this specific method contingent on CLSI receiving further information that satisfies the Subcommittee concerns was made and seconded. Vote: 12 for, 2 against, 0 abstain, 0 absent (Pass)

  - Discussion by AST Subcommittee:
    - Is determination of the level of iron important after chelation?
    - Would like to see data on the agar dilution method with different media manufacturers and different pyridoxal isonicotinoyl hydrazone (PIH) manufacturers
    - EUCAST discourages modifications to the reference method and had not, at the time, reviewed this method yet
    - Modifications to the reference method need to be taken seriously
    - CLSI was cautiously optimistic pending further data
    - CLSI needs to provide clear recommendations to the sponsor
- Identification of potent and specific activity of rifabutin against *A. baumannii*
  - Rifabutin (Mycobutin) is a spiro-piperidyl-rifamycin derived from rifamycin-S, belonging to the class of ansamycin.
  - The antimicrobial activity of the rifamycins is based on their ability to penetrate the bacterial cell wall to inhibit the DNA-dependent RNA polymerase.
  - Rifamycins have limited clinical use on gram-negative infections because of their poor ability to penetrate the gram-negative outer membrane.
  - Rifabutin was identified with potent and specific *in vitro* activity against the gram-negative pathogen *A. baumannii* when tested in nutrient deprived medium.
  - An intravenous (IV) formulation of rifabutin (BV100) has been tested in Phase 2 clinical study (results pending).
- Rifabutin highjacks *A. baumannii* FhuE iron uptake system
  - Rifabutin potent *in vitro* activity is dependent on the TonB-dependent siderophore receptor FhuE that is overexpressed only in iron-limited conditions, leading to strong intracellular accumulation of rifabutin.
- *In vitro* activity in iron-limited conditions correlates with *in vivo* efficacy
  - *In vivo* efficacy of rifabutin has been shown in neutropenic mouse thigh and lung models of *A. baumannii* infection.
  - The 11 strains tested represented 7 strains with an active transport of rifabutin (active FhuE) and 4 strains with an inactive transport of rifabutin (inactive FhuE).
  - The dose of rifabutin required to achieve 1-log10 CFU reduction in thighs or lungs correlated significantly better with the MIC determined in iron-limited medium, indicating that iron-limited conditions are required to determine rifabutin *in vitro* activity that accurately predict *in vivo* efficacy against *A. baumannii*.
- Broth microdilution MIC is not robust
- Broth microdilution conditions tested
  - None of the conditions tested enabled robust MIC determination using the broth microdilution method.

Parameter tested	Variable tested	Outcome
Iron chelators	- PIH - transferrin - deferiprone - bipyridyl	→ All accurately measuring MIC but retaining the multiple skipped wells issue → PIH chosen because non-toxic (MIC > 3500 µM) and widely available
MIC medium	- CAMHB (3 manufacturers) - ID-CAMHB - CAMHB + HS/LHB - MHB - LB - CA-LB	→ All retaining the multiple skipped wells issue or not accurately measuring MIC (CAMHB + HS/LHB)
Agar pre-culture medium	- Blood - MHA - CA-MHA - LB - CHROMagar - RPMI + FCS	→ All retaining the multiple skipped wells issue
Liquid pre-culture medium	- CAMHB - CAMHB + chelator - RPMI + FCS	→ All retaining the multiple skipped wells issue

- Agar dilution leads to clear MIC endpoint
  - Agar dilution results in unambiguous MIC determination.
- Agar dilution in MHA supplemented with PIH accurately measures rifabutin susceptibility
  - Rifabutin *in vitro* activity was tested against a panel of 293 carbapenem resistant *A. baumannii* clinical isolates using the different MIC methods.
  - The MIC distributions were represented by FhuE active (n=223) and FhuE inactive (n=70) strains.
  - Only MIC determined in MHA supplemented with 0.1 mM PIH can resolve the active and inactive FhuE strain populations, which is predictive of *in vivo* potency.
- External validation of the susceptibility testing method
  - The different MIC testing method were evaluated by an external laboratory (IHMA Europe) on a panel of 21 *A. baumannii* strains (15 FhuE active and 6 FhuE inactive strains).
  - BioVersys results were confirmed since only MIC determined in Mueller Hinton agar supplemented with 0.1 mM PIH led to an essential agreement above 90 % and no problem with end point reading.
- Agar dilution MIC are robust across different MHA/PIH lots and manufacturers
  - Different MHA lots and manufacturers were evaluated for MIC determination in the presence of 0.1 mM PIH against a panel of 29 *A. baumannii* strains (19 FhuE active and 10 FhuE inactive strains).
  - Different PIH manufacturers were evaluated for agar dilution MIC determination in MHA supplemented with 0.1 mM PIH against 3 QC candidate *A. baumannii* strains.
  - Agar dilution MIC were robust across the 5 MHA lots and manufacturers tested and across the 6 PIH lots and manufacturers tested.
- Iron quantification by inductively coupled plasma mass spectrometry (ICP-MS)
  - Iron was quantified by ICP-MS in MHA from 11 different lots and manufacturers and in MHA supplemented with 0.1 mM PIH.

- Iron levels vary from 0.5 to 1 mg/kg (9 to 18 µM) across the different lots and manufacturers, which is in line with what was published by EUCAST (21 MHA manufacturers, Åmhan et al. CMI, 2020).
- As expected, PIH supplementation does not significantly affect the level of iron since PIH only chelates free iron in the media.

- Appropriate iron limited condition can be assessed by QC strains
  - Different PIH concentrations were tested for agar dilution MIC determination against a panel of 6 FhuE active *A. baumannii* strains.
  - MICs at 0.1 mM PIH are at the bottom plateau of the sigmoidal curve, indicating sufficient iron chelation and in line with method robustness across different manufacturers.
  - The NCTC 13304 strain (sulbactam-durlobactam QC strain) will be able to control for appropriate iron limited condition during the MIC assay.
- Development of rifabutin MIC test strip device containing PIH
  - Rifabutin MIC test strips (0.002 – 32 mg/L) supplemented with PIH are in development at Liofilchem.
  - MIC test strip containing PIH is a promising agar diffusion testing method to be used as surrogate of agar dilution in clinical laboratories.
- Development of rifabutin disk containing PIH
  - Disk diffusion with or without 100 µg PIH and with varied rifabutin disk contents were tested on FhuE active and inactive strains.
  - As observed with agar dilution MIC, PIH supplementation is required to differentiate FhuE active from FhuE inactive strains.
  - Optimal rifabutin disk potencies, as per CLSI / EUCAST SOP, for disks without and with 100 µg PIH, namely 5 µg and 0.5 µg respectively, were tested against a panel of 16 *A. baumannii* strains (10 FhuE active and 6 FhuE inactive strains).
  - Better correlation of PIH supplemented agar MIC with PIH supplemented disk compared to disk without PIH, confirming that PIH is required to differentiate FhuE active from FhuE inactive strains.
- Summary and Proposal
  - Rifabutin demonstrates potent and specific *in vitro* activity against the gram-negative pathogen *A. baumannii* when tested in iron-limited medium.
  - Potent *in vitro* activity in iron-limited medium is due to active uptake of rifabutin by the *A. baumannii* siderophore receptor FhuE.
  - Iron-limited conditions are required to determine MIC that are predictive of rifabutin efficacy *in vivo*.
  - Agar dilution MIC in the presence of the non-toxic iron chelator PIH is required to determine unambiguous rifabutin MIC.
  - Agar dilution MIC in the presence of 0.1 mM PIH is robust across MHA and PIH manufacturers.
  - Agar diffusion testing methods, such as Liofilchem and disk, are promising surrogates of agar dilution to be implemented in clinical laboratories.
  - Proposal: BioVersys is proposing the agar dilution MIC method using Mueller Hinton agar medium supplemented with 0.1 mM of the iron chelator PIH as reference method for testing rifabutin susceptibility against *A. baumannii*.
- Methods Working Group Discussion and Recommendation
  - Resistance to rifabutin in *Acinetobacter baumannii* is primarily due to fhuE mutation. Can the method detect other resistance mechanisms?
  - RNA polymerase (*rpoB*) mutations have also been observed, and MIC varies by type of mutation (0.016-4 µg/mL in data shown). All FhuE active/*rpoB* mutant strains tested in the in vivo model (all MIC=0.016 µg/mL) showed rifabutin efficacy.
  - Could an fhuE mutation detection test be sufficient to test efficacy of rifabutin vs a phenotypic test? No, as there are *rpoB* mutants that likely confer resistance and rare ADP ribosyltransferase mutants.
  - EUCAST
    - Concerned about *rpoB* mutants and whether enough data has been presented for these. (*rpoB* mutants represent less than 10% of *A. baumannii* strains)

- Could modifying the PIH concentration better separate *rpoB* mutants from wild-type population?
- Does the working group feel confident that this method separates out the wild-type from the non-wild-type?
  - Group would like to see any data that the sponsor can provide at different PIH concentrations.
  - Group would like to see EUCAST data requests and responses.
  - The group would like to standardize method with EUCAST if possible.
- Sponsor's ask: What additional data does CLSI want to see and what is the goal of producing that data? Ideally, the sponsor would like to move forward with a single method for their development work.
- Working Group Next Steps
  - Review discussions between EUCAST and BioVersys
    - Documentation has been provided and will be reviewed at February 2025 Methods Working Group meeting
  - Provide CLSI feedback to sponsor of any additional data needed to move this method forward.

SC DISCUSSION (MAIN POINTS)

- EUCAST stated the company has already shown the lower concentrations of PIH will work. EUCAST requested at one of their meetings that the lowest possible concentration be used if a modification is necessary. Broth microdilution is not the way to go, and agar dilution is method of choice, but they are not convinced that the concentration of PHI is correct. The company has other concentrations that do work, but they have always used 100 µmol, but they have not shown data to prove it is optimal. For *rpoB* mutants, they have not shown that the MIC has value in predicting outcome. If the drug works for all kinds of *rpoB* mutations, then the only test needed is a molecular test, except for the very rare mutants in other genes and they have not shown anything about that yet.
- Concern for a molecular test for FhuE because these receptors are TonB dependent. *A. baumannii* might lose TonB receptor. Are there TonB mutants from the cefiderocol data that could be used to study here?
- These are 6 wild-type strains with active FhuE to illustrate the shift in MIC in the presence of PIH and that 100 µmol PHI allows robust MICs across MHA manufacturers.
- Why does skipping occur in BMD?
  - No definitive answer.
- Are there inner colonies within zones?
  - Inner zone colonies are rare.
  - The 100 µmol is what provides the most robust data.
- Consider a surrogate for testing. If susceptible to X then susceptible to this agent, but if resistant, then can test directly.
- Need to learn from cefiderocol and be rigorous and reproducible upfront.
- Is there data about MIC reproducibility?
  - That might have been presented last meeting
- Question for the sponsor: Is this drug being tested against a range of *Acinetobacter* including CRAB?

COAGULASE NEGATIVE STAPHYLOCOCCI AD HOC WORKING GROUP REPORT

- Goal: Systematically evaluate the performance of antimicrobial susceptibility testing (AST) methods and penicillin-binding protein 2a (PBP2a) immunoassays to detect *mecA/C*-mediated ß-lactam resistance in staphylococci other than *Staphylococcus aureus* (SOSA) (formerly known as CONS)
- Overview of CLSI updates to staphylococcal testing recommendations to predict the presence of *mecA*

Year	Species	Recommendation	Reference
1986	All staphylococci	Publication of methicillin, nafcillin, and oxacillin MIC and DD susceptibility criteria in M100, first informational supplement	NCCLS, 1986
1999	CONS	Establishment of oxacillin MIC and DD breakpoints in M100 that are different than those for *S. aureus*	Tenover *et al.*, 1999 PMID: 10565931
1999	All staphylococci	Deletion of methicillin MIC and DD susceptibility criteria - recommendation to test oxacillin alone	Tenover *et al.*, 1999 PMID: 10565931
2004	*S. aureus*/CONS	Introduction of the cefoxitin disk diffusion test to predict oxacillin resistance	Swenson *et al.*, 2005 PMID: 16081917
2005	*S. lugdunensis*	Inclusion of *S. lugdunensis* with *S. aureus* oxacillin and cefoxitin breakpoints	Swenson *et al.*, 2005 PMID: 16081917
2006	*S. lugdunensis*	Warning that cefoxitin and not oxacillin should be used for disk diffusion or *S. lugdunensis*	Swenson *et al.*, 2005 PMID: 16081917
2012	*S. aureus*	Deletion of oxacillin disk breakpoints	Swenson *et al.*, 2005 PMID: 16081917
2012	CONS	Recommendation to perform cefoxitin disk, PBP2a, or *mecA* test if oxacillin MIC of 0.5-2.0 µg/mL for species other than *S. epidermidis*	Swenson *et al.*, 2005 PMID: 16081917
2014	*S. pseudintermedius*	Publication of oxacillin MIC and disk breakpoints; warning against use of cefoxitin tests for this species	Wu *et al.*, 2016 PMID: 26607988
2015	*S. coagulans/schlieferi*	Publication of oxacillin MIC and disk breakpoints; warning against use of cefoxitin for this species	Huse *et al.*, 2018 PMID: 29187565
2018	*S. epidermidis*	Addition of oxacillin disk test for *S. epidermidis*, confirmation of MIC breakpoint	Naccache *et al.*, 2019 PMID: 31462553
2021	SOSA (CONS)	Oxacillin breakpoint updated	Humphries *et al.*, 2020 PMID: 33115842

- *Staphylococcus saprophyticus* isolates included in the study

Institution	Year of collection	Number of isolates		Country of origin	Specimen source
		***mecA* negative**	***mecA* positive**		
IHMA	2013-2020	14	6	Philippines, Hungary, Brazil, Kuwait, United States, Portugal, Netherlands, Israel, Russia, South Africa, Belgium, Venezuela, Nigeria	Abscess, blood, urine, peritoneal fluid, cellulitis, skin ulcer, endotracheal aspirate, sputum
CHLA	Unknown	2	2	United States of America	Urine, peritoneal fluid
BJMC	2018-2020	5	8	United States of America	Urine, blood
UA (Alberta)	Unknown	2	4	Canada	Blood, bone chip, urine
VUMC	Unknown	6	2	United States of America	Blood
WCMC	2021	1	1	United States of America	Urine, wound
Total (53 isolates)		**30**	**23**		

Abbreviations: BJMC, Barnes Jewish Medical Center; CHLA, Children's Hospital Los Angeles; IHMA, International Health Management Associates; UA, University of Alberta, VUMC, Vanderbilt University Medical Center; WCMC, Weill Cornell Medical Center

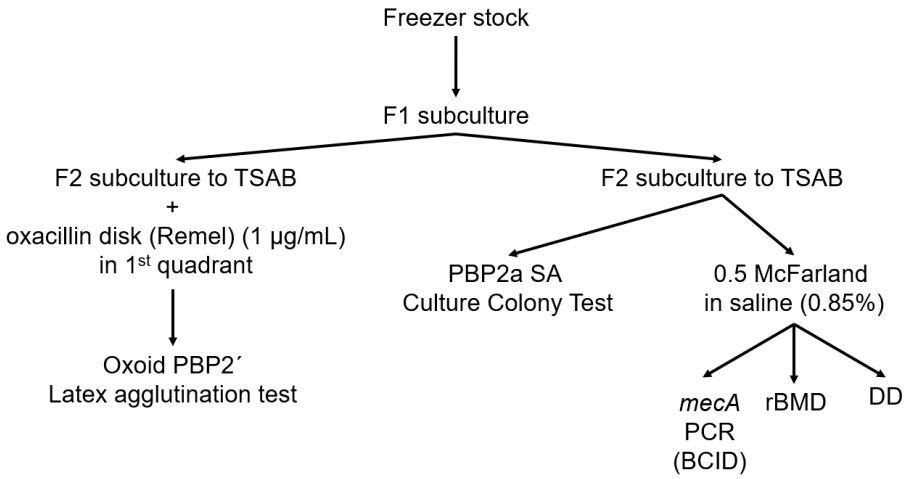
- Reagents and methods
  - Reference method: *mecA* PCR (BCID panel, bioMérieux, Inc.) (all isolates *mecC* negative)
  - Reference broth microdilution (rBMD): frozen-form Sensititre custom panels:
    - Three different Cation-Adjusted Mueller-Hinton broth (CAMHB) manufacturers:
      - Difco
      - BBL
      - Oxoid
    - Cefoxitin (Thermo Fisher Scientific) range: 0.015-32 µg/mL (read between 16-20 h)
    - Oxacillin (Toku-E) + 2% (w/v) NaCl range: 0.015-32 µg/mL (read at 24 h)
    - 3 oxacillin MIC datapoints for each isolate (1 per CAMHB manufacturer)
    - 3 cefoxitin MIC datapoints for each isolate (1 per CAMHB manufacturer)
  - Disk diffusion (DD; read oxacillin between 16-18 h; read cefoxitin at 24 h):
    - Three different Mueller-Hinton agar (MHA) manufacturers:
      - Becton, Dickinson and Company
      - Hardy Diagnostics
      - Remel (Thermo Fisher Scientific)
    - Three different cefoxitin (30 µg) and oxacillin (1 µg) disk manufacturers:
      - Becton, Dickinson and Company

- Hardy Diagnostics
- Remel (Thermo Fisher Scientific)

▪ 9 oxacillin zone size datapoints for each isolate (3 different disk manufacturers and 3 different MHA manufacturers)
▪ 9 cefoxitin zone size datapoints for each isolate (3 different disk manufacturers and 3 different MHA manufacturers)

○ PBP2a immunoassays:
▪ Oxoid PBP2´ Latex Agglutination Test (Thermo Fisher Scientific)
▪ Clearview PBP2a SA Culture Colony Test (lateral flow immunoassay) (Abbott)

- Workflow



- CLSI M100-S34 breakpoints evaluated in this study

Breakpoint description	Susceptible breakpoint	Resistant breakpoint	Abbreviation
Oxacillin MIC, *S. aureus*/*S. lugdunensis*	≤2 µg/mL	≥4 µg/mL	OX MIC SA/SL
Oxacillin MIC, *Staphylococcus* species other than *S. aureus*/*S. lugdunensis*	≤0.5 µg/mL	≥1 µg/mL	OXA MIC STAPH
Oxacillin zone diameter, *S. epidermidis*, *S. pseudintermedius*, *S. coagulans*, *S. schleiferi*	≥18 mm	≤17 mm	OX DD SE/SC/SP/SS
Cefoxitin MIC, *S. aureus*/*S. lugdunensis*	≤4 µg/mL	≥8 µg/mL	FOX MIC SA/SL
Cefoxitin zone diameter, *S. aureus*/*S. lugdunensis*	≥22 mm	≤21 mm	FOX DD SA/SL
Cefoxitin zone diameter, *Staphylococcus* species other than *S. aureus*, *S. lugdunensis*, *S. pseudintermedius*, *S. coagulans*, *S. schleiferi*	≥25 mm	≤24 mm	FOX DD STAPH

- Overview of *S. saprophyticus* results

Method	Antimicrobial	Breakpoint	Susceptible	Resistant	Categorical agreement (%)	Very major error (%)	Major error (%)
rBMD	Oxacillin	OX MIC SA/SL	≤2 µg/mL	≥4 µg/mL	88.1 (140 results/159 results)	27.5 (19 results/69 results)	0 (0 results/90 results)
rBMD	Oxacillin	OX MIC STAPH	≤0.5 µg/mL	≥1 µg/mL	91.8 (146 results/159 results)	5.8 (4 results/69 results)	10 (9 results/90 results)
DD	Oxacillin	OX DD SE/SC/SP/SS	≥18 mm	≤17 mm	56.8 (271 results/477 results)	3.9 (8 results/207 results)	73.3 (198 results/270 results)
rBMD	Cefoxitin	FOX MIC SA/SL	≤4 µg/mL	≥8 µg/mL	78 (124 results/159 results)	46.4 (32 results/69 results)	3.3 (3 results/90 results)
DD	Cefoxitin	FOX DD SA/SL	≥22 mm	≤21 mm	86 (410 results/477 results)	32.4 (67 results/207 results)	0 (0 results/270 results)
DD	Cefoxitin	FOX DD STAPH	≥25 mm	≤24 mm	88.1 (420 results/477 results)	14 (29 results/207 results)	10.4 (28 results/270 results)
DD	Oxacillin	EUCAST v14.0 (SC/SP/SS, *S. intermedius*)	≥20 mm	<20 mm	52 (248 results/477 results)	0.5 (1 result/207 results)	84.4 (228 results/270 results)

- Categorical agreement = number of categorical result matches/total number of results
- Very major error = number of very major error results/total number of resistant results by reference method
- Major error rate = number of major error results/total number of susceptible results by reference method

- Comparison of *mecA* results with CLSI M100-S34 oxacillin MIC breakpoints for SOSA

Species	Percentage		Reference
	M100-S34		
	VME at ≤0.5 µg/mL	ME at ≥1.0 µg/mL	
S. capitis	0	0	Humphries *et al.*, 2020 PMID: 33115842
S. coagulans/schleiferi	0	0	Huse *et al.*, 2018 PMID: 29187565
S. epidermidis	0	2.0	Naccache *et al.*, 2019 PMID: 31462553
S. haemolyticus	6.4	1.4	Humphries *et al.*, 2020 PMID: 33115842
S. hominis	5.0	0	Humphries *et al.*, 2020 PMID: 33115842
S. pseudintermedius	0	0	Wu *et al.*, 2016 PMID: 26607988
S. saprophyticus	5.8	10	This work
S. warneri	0	1.3	Humphries *et al.*, 2020 PMID: 33115842

- Performance of PBP2a immunoassays

FDA approved for *S. aureus* and SOSA		Oxoid PBP2´ Latex Agglutination Test	
		Negative	Positive
BCID *mecA* PCR (gold standard)	Negative	30 (TN)	0 (FP)
	Positive	3 (FN)	20 (TP)

- Sensitivity, 87% (95% confidence interval [CI] 66.4-97.2%)
- Specificity, 100% (95% CI %)

FDA approved for *S. aureus* only		PBP2a SA Culture Colony Test	
		Negative	Positive
BCID *mecA* PCR (gold standard)	Negative	29 (TN)	1 (FP)
	Positive	0 (FN)	23 (TP)

- Sensitivity, 100% (95% CI 85.2-100.0%)
- Specificity, 96.7% (95% CI 82.8-99.9%)

- False-positive PBP2a SA culture colony test result
  - *mecA* PCR negative (multiple methods)
  - *mecC* PCR negative
  - OX MIC:
    - Difco, 0.5 µg/mL
    - BBL, 0.5 µg/mL
    - Oxoid, 1 µg/mL

FDA approved for *S. aureus* only		PBP2a SA Culture Colony Test	
BCID *mecA* PCR (gold standard)		Negative	Positive
	Negative	29 (TN)	1 (FP)
	Positive	0 (FN)	23 (TP)

- Sensitivity, 100% (95% CI 85.2-100.0%)
- Specificity, 96.7% (95% CI 82.8-99.9%)

- Summary
  - No single AST method/CLSI breakpoint accurately differentiates between *mecA*-negative and *mecA*-positive *S. saprophyticus* isolates
  - The AHWG does not endorse proposing oxacillin or cefoxitin disk diffusion or BMD breakpoints for *S. saprophyticus*
  - Immunoassays for PBP2a appear to be the best method for detecting *mecA*-mediated β-lactam resistance in *S. saprophyticus* (should only be performed for serious infections, not isolates recovered from the urinary tract)
  - CLSI should continue to endorse tests that detect *mecA* or PBP2a as the definitive methods for detecting *mecA*-mediated β-lactam resistance in SOSA (certainly for isolates that are not *S. coagulans*, *S. epidermidis*, *S. pseudintermedius*, or *S. schleiferi*)
- Methods Working Group Discussion and Recommendation
  - When CLSI did the addition of salt to MHB for better detection of *mecA* in *Staphylococcus aureus*, this was not done for SOSA – something to be considered?
  - Have you looked at changing the MIC/zone diameter breakpoints to see if there is a better cutoff for separating *mecA*-positive from *mecA*-negative strains? Not yet.
  - Concern about BCID being the right method for determining *mecA* status in these isolates.
  - Does EUCAST have data? Yes.
  - A motion was put forward to remove the recommendation of oxacillin MIC/cefoxitin disk for *S. saprophyticus* from the CLSI M100 and recommending *mecA*/PBP2a testing only, but the motion was not seconded in favor of further data generation and analysis.
  - Next steps:
    - Determine *mecA*/*mecC* status of isolates by PCR or WGS to definitively confirm *mec* status.
    - Review EUCAST data.
    - Generate bar charts to visualize MIC and disk diffusion distributions to see if there is a better breakpoint for separation of *S. saprophyticus* and *mecA* presence/absence.

SC DISCUSSION (MAIN POINTS)

- When CLSI added salt to MHB for *S. aureus* for better detection of *mecA*, this was not done for SOSA, so should this be considered?
  - Clarification of the above statement: It was adding salt to cefoxitin for SOSA, not oxacillin.
- Was the same inoculum used for AST and PCR?
  - Think so but are not sure.
- Suggest using the Cepheid assay for *mecA*, so that might be another method.
- There were 2 isolates that were PBP2a positive and *mecA*-negative on the BioFire, which raises concerns if *mecA* on the BioFire is the appropriate method to check for *mecA* resistance.
- Do clinical laboratories need to test for *mecA* in *S. saprophyticus*?
- Does the ECUAST data conflict with CLSI data?
  - EUCAST would like to see the raw data from CLSI. The EUCAST data has only a few *mecA*-positive isolates and the data is old.
  - Romney Humphries has data that shows the *S. saprophyticus* wild-type distribution matches EUCAST, but the disk diffusion data shows the breakpoints do not work.
- Overall feeling that the oxacillin and methicillin have been stressed as far as they can go, and the field should be using PBP2a antigen testing instead.
- There is interest from numerous clinical microbiologists to meet with the manufacturer to get a clinical indication for SOSA.

REVISIONS TO CLSI M100 TABLE 6A

- Objective: Other than newer agents the information in Table 6A for specific agents, especially generic agents, has not been updated. The goal is to update the table with recent, useful, relevant information as may be available for any agent listed in the table.
- Process: The table will be provided in an Excel file to institutions that are experienced in making BMD MIC panels. A separate column will be created for each contributing institution. They will be instructed to record comments next to select agents that may be additional information not shown in the current table or perhaps is contrary to what is in the table. It is expected that further clarification and inquires may be required from the contributors, after which modifications will be made to the table and it will then be reviewed by the Methods Working Group.
- Table Example

Antimicrobial Agent	CAS Registry Number	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.	Notes: Add current footnotes (except footnotes a-d, which will be footnotes at end of table)	CDC Amelia/David comments	LSI comments
Amikacin (Amikacin disulfate salt)	39831-55-5	Water	Water			
Amoxicillin	26787-78-0	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L	This make take time to go into solution (20-30 minutes mix). Best to make on day of panel pour, as it tends to precipitate when frozen at concentrations >1,000 µg/mL.		do not experience it taking 20-30 mins to mix
Ampicillin (trihydrate)	7177-48-2	Phosphate buffer, pH 8, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L			
Avibactam (sodium)	1192491-61-4	Water	Water			
Azithromycin	117772-70-0	95% ethanol or glacial acetic acid[a,c]	Broth media	Recommend to make on day of pour. All serial dilutions must be done with CAMHB or broth being used for panel pour, not water.		serial dilutions are always done in CAMHB
Azlocillin		Water	Water		CDC does not test	
Aztreonam	78110-38-0	Saturated ~~solution~~ sodium bicarbonate solution	Water	To make saturated sodium bicarbonate solution: Add ~10 g of NaHCO3 into 100 mL of d-water. Stir and warm if needed to clear the solution; then filter sterilize.		
Besifloxacin		Methanol	Water		CDC does not test	
Biapenem		Saline[d]	Saline[d]		CDC does not test	
Cadazolid		DMSO[a]	Water or broth		CDC does not test	
Carbenicillin		Water	Water		CDC does not test	
Cefaclor		Water	Water		CDC does not test	
Cefadroxil		Phosphate buffer, pH 6, 0.1 mol/L	Water		CDC does not test	
Cefamandole		Water	Water		CDC does not test	
Cefazolin (sodium salt)	27164-46-1	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L			
Cefdinir		Phosphate buffer, pH 6, 0.1 mol/L	Water		CDC does not test	
Cefditoren		Phosphate buffer, pH 6, 0.1 mol/L	Water		CDC does not test	
Cefepime (HCl)	123171-59-5	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L or water		We do not use water in our procedure	We do not use water
Cefetamet		Phosphate buffer, pH 6, 0.1 mol/L	Water		CDC does not test	

- Laura Koeth's Call for Help
  - I do have a good idea of possible "MIC PLATE MAKER" contributors, however, I definitely don't know you ALL…please reach out to me if your institution is willing and able to participate.
  - Also, I welcome any help in the process…hopefully, we will get plenty of input and when that occurs, decisions regarding specific wording, questions to contributors and/or outside referrals may be needed and assistance with these activities would be greatly appreciated. Please let me know if you can assist in any way.
  - Email me at: lkoeth@labspec.org
- Proposed Timeline
  - Finalize List of Contributing Institutions and Contacts: immediately following January 2025 meeting
  - Send the table to Contributing Institutions: February 2025

- Request input by mid-April 2025
- Present updated table to Methods Working Group: June 2025

SC DISCUSSION (MAIN POINTS)

- Consider what is needed to accept a different solvent?
- Ceftazidime is difficult with the official CLSI method. Many people are not actually following the exact table method for reference method BMD.
- The data around the compounds and solvents was not clear in CLSI M100 originally, which has led to laboratories having variations like different solvents.

INTRINSIC RESISTANCE DEFINITION AD HOC WORKING GROUP REPORT

- Current Intrinsic Resistance Definition

Appendix B. Intrinsic Resistance

Intrinsic resistance is defined as inherent or innate (not acquired) antimicrobial resistance, which is reflected in wild-type antimicrobial patterns of all or almost all representatives of a species. Intrinsic resistance is so common that susceptibility testing is unnecessary. For example, *Citrobacter* spp. are intrinsically resistant to ampicillin.

These tables can be helpful in at least three ways: 1) they provide a way to evaluate the accuracy of testing methods; 2) they aid in the recognition of common phenotypes; and 3) they can assist with verification of cumulative antimicrobial susceptibility test data. In the tables, an "R" occurring with an antimicrobial agent–organism combination means that strains should test resistant. A small percentage (1% to 3%) may appear susceptible due to method variation, mutation, or low levels of resistance expression.

Each laboratory should decide which agents to test and report in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. If tested, the result for an antimicrobial agent–organism combination listed as having intrinsic resistance should be reported as resistant. Consideration may be given to adding comments regarding intrinsic resistance of agents not tested. See Appendix A, footnote a.

- Proposed Intrinsic Resistance Definition

  Intrinsic resistance (IR) is defined as inherent or innate (not acquired) antimicrobial resistance, evidenced by high MIC or reduced zone diameter values for specific antimicrobial agent-organism combinations for all or nearly all isolates of a microbial species or organism group. The MIC distribution for antimicrobial agent-organism combinations exhibiting IR generally displays a high modal MIC well above the expected clinically achievable antimicrobial concentrations.

  IR also includes antimicrobial agent-organism combinations for which available PK/PD data show insufficient antimicrobial exposure or when available clinical data demonstrate lack of efficacy.

  Susceptibility testing is unnecessary for organisms considered intrinsically resistant to an antimicrobial. However, if testing is performed, they should be reported as resistant or intrinsically resistant. MIC and zone diameter values should not be reported as some isolates may exhibit low MIC or wide zone diameter values due to method variation, mutation or low levels of resistance gene expression.

  - Note: For specific documents, each group would include examples of IR with the components of the new definition.
- IR Definition AHWG Discussions

- Noted numeric % (≥97%) value to be considered IR was no longer part of the definition
- AHWG thought the data was not robust enough to decide on a percentage
- EUCAST has replaced intrinsic resistance with:
  - Expected Susceptible Phenotype- the wild-type should be considered susceptible (S or I) to the agent and a very high proportion (99%) of isolates should be devoid of acquired resistance to the agent
    - Group A streptococci and penicillin
  - Expected Resistant Phenotype- 90% or more should be considered resistant
    - *Klebsiella* and ampicillin
- EUCAST uses these definitions to help with identification confirmation
- EUCAST does not mention PK/PD in their definition
- Evaluating MIC results without a defined % cutoff and including the PK/PD component in the definition makes applying the definition difficult.
- M45 organisms: Issues with organisms where most MICs are high and fall above achievable therapeutic concentrations, but are not IR. Are these now IR based on this definition or something else?
- PK/PD should be included in the IR definition especially for clinical treatment failures (eg, *Pseudomonas aeruginosa* and gentamicin).
- Discussion around examples of organisms that will fit into this definition.
- Antifungal AST Subcommittee is working on a definition for reduced susceptibility which is separate from intrinsic resistance which was not discussed in detail.

- Methods Working Group Discussion and Recommendation
  - Could not reach consensus on the proposed definition.
  - Final decision: For the AHWG to add background and history of the definition and add examples for further clarification. Test example organisms and determine how they stand up to the definition with both the MIC and PK/PD data included without a percentage in the definition.

SC DISCUSSION (MAIN POINTS)

- Check with Barb Zimmer on the previous discussions and notes on intrinsic resistance.
  - AHWG is already working with Barb Zimmer.
- Consider the modal MIC and if PK/PD data says treatment is not achievable or if there is clinical data.
- Be careful about drugs that are inferior vs not active. One example is *P. aeruginosa* and gentamicin, it is not a good idea to give gentamicin to someone with a *P. aeruginosa* infection, but it is thought by some to be better than placebo.
- If PK/PD data is included in the detection, need to consider site specific infections.
- There are lots of issues with PK/PD because it varies based on the patient population.
- It should be independent of body site; the drug should never work.
- Consider two definitions, one for a property of the organism vs the drug does not work clinically and should be treated as if resistant.
- Do not make this too complicated for laboratories. Consider putting this in the CLSI M23.

COLONY COUNT CONSIDERATIONS

- Should CLSI / EUCAST “reference” and/or “standard” methods provide more guidance for colony counts?
  - Some have adopted protocols to get colony counts to “fit” desired CFU

- May be useful for those with a limited understanding of the range of colony counts that are obtained from a McFarland 0.5 suspension based on the organism
- Could be an opportunity for further harmonization between CLSI and EUCAST methods

- Key Points
  - CLSI M07 and M02
    - Includes procedure and provides range for 0.5 McFarland and final inoculum based on CFU/ml
    - No mention of varying colony counts by species
    - Target range only for *E. coli* ATCC 25922
  - CLSI M23
    - Colony count averages are included in Tier 2 QC studies report but only reviewed if troubleshooting issues with variability
    - Potential to compile data to establish colony count ranges for other organisms
  - CLSI M100 – references E. coli ATCC for troubleshooting
  - EUCAST - defers to ISO 20776-1
  - ISO 20776-1
    - Extrapolates range to organisms other than *E. coli* ATCC 25922
    - Section 4.4 describes "adjusting" inoculum to meet colony count range
  - FDA - requires colony counts when diagnostic manufacturers submit data for FDA approval of AST devices
  - Numbers of CFUs in McFarland 0.5 suspension may vary based on the organism size/density
  - Some adjust 0.5 McFarland suspension turbidity to high end when colony count is likely to be low and to low end when colony count is likely to be high
- Small Informal Survey of CLSI AST Participants
  - Do you adjust 0.5 McFarland suspensions based on organism size/density?
  - Very limited responses but approach varies:
    - Some adjust inoculum, some don't
    - If adjusting, stays within defined range for 0.5 McFarland standard.
  - Methods of adjustment
    - Adjust for *S. pneumoniae* and *C. difficile*
      - Stay within 0.5 McFarland range on turbidity meter
      - Strive to use 18-20 hour cultures (preferably 18 hour) and adjust to high end of range
      - Improves colony counts but trend may still be lower than *E. coli* ATCC 25922
    - Adjust turbidity to meet colony counts for *E. coli* ATCC 25922
    - Per internal SOP based on organism/organism group tested, adjust to higher or lower end of 0.5 McFarland range
    - Transfer a larger volume from 0.5 McFarland suspension to broth for several fastidious organisms (eg, Streptococci, *Corynebacterium, Aerococcus, Kingella kingae* and *Campylobacter*)
- Potential Impact of Inoculum Suspension Protocol Variability
  - Tier 2 QC ranges - if adjustments are made for QC study but not by user, could impact in-range results
  - Comparing results from various sources in research settings
    - MIC distributions (eg, scattergrams when setting breakpoints, ECOFFs/ECVs, MIC 50/90 from pharmaceutical studies)
  - Method to method comparison studies

- Likely use same inoculum suspension for reference and test
  - Results from testing in clinical settings
    - Use of McFarland 0.5 standard
    - Use of various types of photometric devices
  - Do various CFU/ml within "acceptable 0.5 McFarland range" impact zones/MICs?
- ISO AST Documents
  - ISO 20776-1:2019
    - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Part 1: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases
  - ISO 20776-2:2021
    - Clinical laboratory testing and in vitro diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Part 2: Evaluation of performance of antimicrobial susceptibility test devices against reference broth micro-dilution
  - ISO 20776-3: - under development
    - Clinical laboratory testing and in vitro diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices Part 3: Disc-diffusion agar reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases
  - ISO/TS 16782:2016
    - Clinical laboratory testing — Criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing
- Potential Next Steps
  - More formal/larger survey to assess variability in inoculum preparation practices
  - Compile data for other organisms to establish target colony count ranges (eg, Tier 2 QC Studies, commercial AST manufacturer studies)
  - Additional guidance to align practices
    - Future CLSI M02 and M07 revisions
    - White paper, FAQs
    - Future education from Outreach Working Group
    - Revision to ISO 20776-1
- Methods Working Group Discussion and Recommendation
  - What is the ask? Ultimate question is whether various CFU/ml within an acceptable 0.5 McFarland/inoculation impact results?
  - Ask for AHWG to review data to determine if this is an issue
  - Data that already exists: Earlier studies from 1970s showed most were equivalent (+/- dilution) within a log, study using QC data, previous CLSI presentations with similar data
  - Data should include contemporary isolates and anaerobes. Does this already exist?
  - Examples of adjusted McFarland currently used in laboratories: Mucoid colonies
  - Where does colony count make a difference and focus on a guidance for these areas
  - Support for continued work to review data already available but AHWG not needed at this time
  - Present again with specific objectives

SC DISCUSSION (MAIN POINTS)

- This would be better for the CLSI M02 and M07.
- There was surprise that laboratories are making adjustments. It is helpful to know if this truly is an issue or is this something to clarify with ISO standards updates. CLSI has not established colony count ranges for organisms other than *E. coli* QC. The Tier 2 QC studies have colony counts, so the data is available. *S. pneumoniae* might have some non-viable cells.

GUIDANCE FOR REFERENCE MIC METHOD DEVIATIONS

- Background
  - New antimicrobial agents are urgently needed.
  - New agents in development often include those with:
    - Novel mechanisms of action
    - Non-traditional targets
    - Different properties than existing antimicrobials
  - Reference method AST may not be suitable because:
    - MICs too difficult to read, erratic, or non-reproducible
    - MICs do not correlate with clinical efficacy
    - Current media components may impact activity of the antimicrobial
  - At present:
    - No official guidance is available for these situations
    - CLSI and EUCAST follow independent validation processes
- FDA Position

- Reference MIC Method
  - CLSI methods are based on:
    - M07 (1st ed. 1980)
      - Was a precursor to ISO 20776-1 (see below)
    - M23 (1st ed. 1986)
    - ISO 20776-1 (1st ed. 2006)
  - EUCAST methods are based on ISO 20776-1
  - Both CLSI and EUCAST use cation-adjusted Mueller Hinton broth (CAMHB).
  - But CLSI and EUCAST do not always strictly follow 20776-1.
    - Some deviations are well established
    - Some deviations have been approved more recently
    - Others are still under discussion
- Well-established MIC method deviations
  - CLSI and EUCAST use different media for testing fastidious bacteria
    - Although CLSI is harmonizing MIC testing with EUCAST for *Haemophilus* spp. and *Streptococcus pneumoniae* for some antibiotics
  - Agar dilution for testing *Neisseria* spp.
  - Antimicrobial-specific deviations already established:
    - Daptomycin – Ca^{2+} supplementation to 50 mg/L
    - Oxacillin - 2% NaCl supplementation
    - Mecillinam and fosfomycin - agar dilution only
    - Fosfomycin – glucose-6-phosphate supplementation
    - Oritavancin/telavancin – 0.002% polysorbate-80 supplementation
    - Omadacycline/tigecycline- fresh media
- Recently approved AST deviations
  - Cefiderocol - Iron-depleted CAMHB (CLSI and EUCAST)
    - CLSI reviewing the need for additional medium controls
  - Exebacase - CAMHB + 25% horse serum and 0.5 mM DL-dithiothreitol [in 5% CO_2 for non-*S. aureus*] (CLSI only)
  - Zosurabalpin – CAMHB + 20% heat-inactivated horse serum (CLSI only)
- Recently non-approved AST deviation
  - OMN6
    - FDA granted fast-track designation
    - FDA approved a planned Phase II study Nov 2023
    - *In vitro* activity inhibited by the high cation levels in CAMHB
    - Testing in MHB with total Ca^{2+} and Mg^{2+} concentration ≤15 mg/L (NA-MHB) was proposed based on a better correlation with *in vivo* model data
    - Data presented to CLSI QC Working Group in June 2023, but use of this modified method was not approved.
- Why is harmonized guidance so important?
  - Avoids invalid pre-IND data generation.
    - Either using a non-reference method inappropriately or not considering the key features for selecting a 'new' method.

        - Data packages may need to be repeated costing time and money
    - Troubleshooting for potentially problematical molecules.
        - Outlines what might trigger the need to develop a new method.
    - Defines a process and types of evidence required to support any deviation.
        - Validated by both CLSI and EUCAST
- Who are the stakeholders?
    - Drug developers (incl. BEAM Alliance)
    - Funding sources: CARBX, INCATE
    - Standardization bodies: ISO, CLSI, EUCAST
    - Regulators: EMA, FDA
    - AST device manufacturers
- Proposed next steps
    - Set up a joint CLSI/EUCAST AHWG within CLSI Methods Working group like the working group successfully used to harmonize disk mass.
    - A Novel/Emerging AST Methods Ad Hoc Working Group was proposed at the June 2024 CLSI meeting.
    - Invite representatives of the key stakeholders to join or at least participate.
- Methods Working Group Discussion and Recommendation
    - Goal is not to deviate because it is easier but because it is needed and should be reserved for when it is absolutely needed
    - Manufacturers deviate during FDA step before considering AST testing and discussing with CLSI/EUCAST; Can FDA point to a CLSI/EUCAST guidance
    - Need a guidance early on so that manufacturers know when they should think about deviating from the reference
    - Where would a guidance go? CLSI M23, M07 or supplement?
    - Motion to create an AHWG to include EUCAST to provide guidance to companies when they want to deviate from the reference method. WG Vote: 8-0-1-3.

SC DISCUSSION (MAIN POINTS)

- A joint effort between CLSI and EUCAST to help ensure the same process is needed.
- Which working group should oversee this AHWG?
    - Consensus is that this should be an AHWG under the Joint CLSI EUCAST Working Group.
- Need to advertise to reach companies early.
- EUCAST has a document with who companies should contact and what data is needed.
- CLSI needs to be realistic that old methods might not work for these new compounds. Need to be more flexible in accepting new methods.
- There was a concern about the buzz of having lower MICs and modifying methods just to have a lower MIC.
- Need guidelines on when changes need to be made.
- CLSI needs to clarify the data needed to demonstrate that the reference method does not work.
- Commercial companies are concerned about publicly sharing their data, so they have concerns about coming to CLSI early on. Could CLSI and EUCAST provide initial feedback in a less public way?
    - There will be proprietary data if this is early in drug development.
    - CLSI has in the past put together an abbreviated packet of information for public distribution and more detailed, sensitive data.

- Making rules is important, could use the following: 1) Is there a biological reason reference method media does not work? 2) Does the reference method not clearly separate wild-type from resistance? and 3) *in vivo* animal models to demonstrate the reference method does not work and the altered method does work.

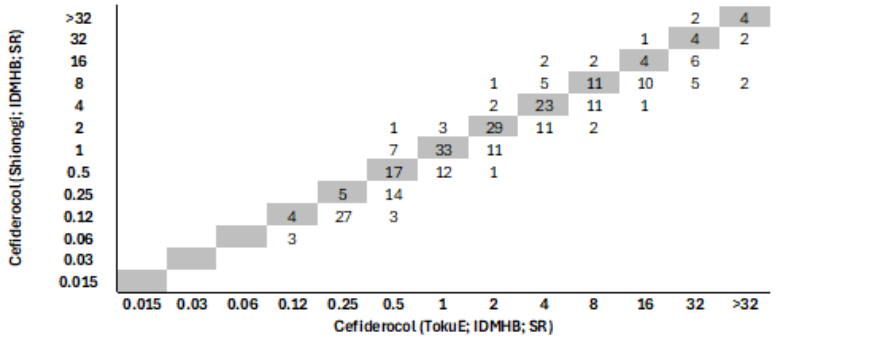
A motion to form an ad hoc working group under the Joint CLSI EUCAST Working Group to provide guidance on deviations from the reference method was made and seconded. Vote: 12 for, 0 against, 0 abstain, 2 absent (Pass)

CEFIDEROCOL AD HOC WORKING GROUP REPORT

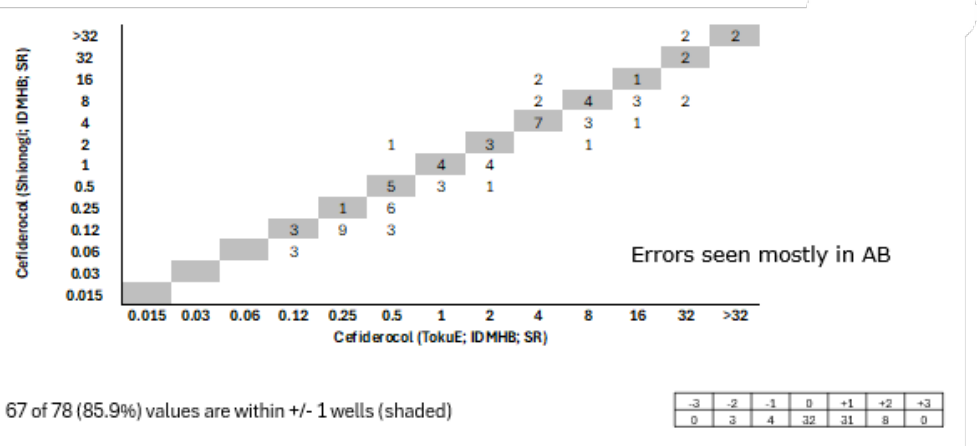
- Charge: Reproducible means of testing cefiderocol by broth microdilution or disk diffusion for Enterobacterales, *P. aeruginosa*, *Acinetobacter*, *S. maltophilia*, and appropriate quality control to ensure testing method is accurate.
- Source of Cefiderocol Powder
  - Laboratories should use pure powder for susceptibility testing and not patient vials
  - Pure powder is costly
  - Solvents are different between pure powder and patient vials (DMSO vs water)
  - Shionogi currently provides patient vials but are transitioning to providing pure powder
  - Parallel testing between pure powder and Shionogi patient vials
    - Data showing equivalency
    - EUCAST and Shionogi
- Cefiderocol Powder Supply
  - Cefiderocol (active pharmaceutical ingredient (API)) is commercially available through several vendors
    - MedChem Express, Sigma, MedKoo, AdooQ, BioSynth, Toku-E
    - Soluble in DMSO
  - Shionogi provides cefiderocol commercial drug product (DP), not API, for Investigator Initiated Research (IIR) studies and to diagnostic companies, CDC, and JMI (surveillance studies)
    - DP is easier to handle and store (4ºC, multiple years, better solubility in aqueous solutions)
    - DP contains additional excipients (sucrose, NaCl)
    - Excipients do not interfere with MIC measurements
    - Equivalency of DP and API demonstrated for MIC measurements using carbapenem-resistant Enterobacterales
    - Test with certain combinations and additional species ongoing
  - Shionogi is exploring provision of API if deemed necessary
- Cefiderocol Shionogi versus Commercial Source
  - Study conducted by Element/JMI
  - Testing
    - MIC testing
      - Cefiderocol (dilution range 0.03 to 64 µg/mL)
      - BBL MHB – iron-depleted with Chelex
      - 2 powder sources Shionogi patient vial and Toku-E - same inoculum for both
    - MIC reading
      - Significant reduction (SR) according to CLSI M100 Ed33 (did not use new guidance from CLSI M100 Ed34)

- Isolates
  - Organisms included: *Acinetobacter baumannii-calcoaceticus* species complex (31), *Citrobacter freundii* species complex (1), *Enterobacter cloacae* species complex (10), *Escherichia coli* (12), *Klebsiella aerogenes* (6), *K. pneumoniae* (18), *Proteus mirabilis* (1), *Pseudomonas aeruginosa* (25), and *Serratia marcescens* (2).
  - 75% carbapenem-resistant
  - Includes 20-25 replicates of QC testing

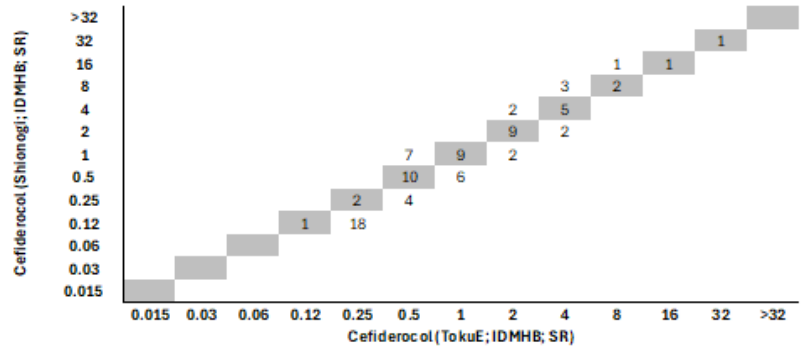
- All gram-negative isolates



- *A. baumannii-calcoaceticus* complex isolates



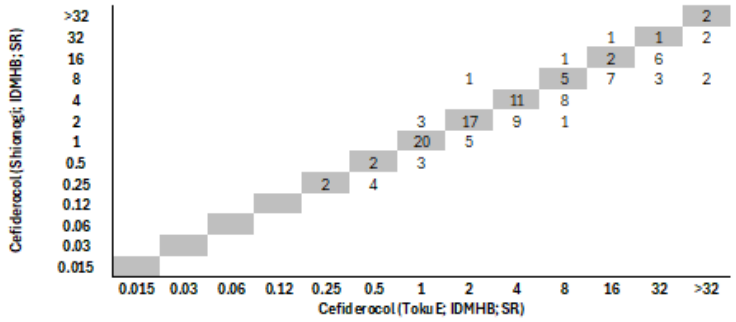
- *P. aeruginosa*



85 of 85 (100%) values are within +/- 1 wells (shaded)

-3	-2	-1	0	+1	+2	+3
0	0	13	40	32	0	0

- Enterobacterales



111 of 118 (94.1%) values are within +/- 1 wells (shaded)

-3	-2	-1	0	+1	+2	+3
0	4	22	134	107	12	2

- Quality Control Data

Strain	Cefiderocol	No. of occurrence at MIC (mg/L)											
		0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32
P. aeruginosa ATCC 27853	Shionogi				18								
P. aeruginosa ATCC 27853	TokuE				1	17							
P. aeruginosa SR27001	Shionogi						4	13					
P. aeruginosa SR27001	TokuE						10	7					
K. pneumoniae ATCC BAA-2814	Shionogi							14	4				
K. pneumoniae ATCC BAA-2814	TokuE							12	6				
A. baumannii NCTC 13304	Shionogi			3	14	1							
A. baumannii NCTC 13304	TokuE				6	10	2						

Chelation QC

Showed most variability

The expected cefiderocol MIC range for *P. aeruginosa* SR27001 based on historical data is 0.5 – 4 mg/L and it was used as an additional internal control (shaded in grey).

- MHB Variability and Time to Chelation
  - Differences in media manufacturers even with sufficient iron depletion
  - Unable to recommend specific media manufacturers
  - Shionogi working on a manuscript to guide users on preferred manufacturers and observed differences in manufacturers
  - Additional recommended modifications to the method by AHWG in CLSI M100 Appendix H
- Proposed Appendix H Changes

H1.2 Iron-Depleted Cation-Adjusted Mueller-Hinton Broth[a]

The steps for preparing iron-depleted cation-adjusted Mueller-Hinton broth (ID-CAMHB) are listed below.[2]

Step	Action	Comments
1	Prepare the CAMHB. or MHB.	Follow manufacturer's instructions.
2	Autoclave the media and let cool to room temperature.	
3	Add 100 g chelating resin to 1 L autoclaved CAMHB.[2] or MHB (based on step one).	Removes polyvalent metal cations in the medium- to low-level concentrations (range, 0–0.18 mg/L).[2]
4	Stir the solution at room temperature for approximately 6 h using a magnetic stir bar.	
5	Filter the solution using a 0.2-µm filter.	Removes the resin. It is recommended that testing for residual iron levels of the filtrate should be conducted at this step to confirm that the iron content does not exceed 0.03 mg/L. Residual iron content can be measured with a commercial iron detection kit capable of detecting low levels of iron (0.02 mg/L). If iron levels exceed 0.03 mg/L, restart the procedure at the chelation step 3 above.
6	Check the pH to determine whether it is 7.3 ± 0.1.	If the pH is above 7.4, adjust it using 1 or 6 N HCl (use of 6 N HCl will minimize the volume required to adjust the pH). If the pH is below 7.2, use 2.5 N NaOH.
7	Add the cation to achieve final concentrations in the following ranges: • Ca^{2+} 20–25 mg/L • Mg^{2+} 10–12.5 mg/L • Zn^{2+} 0.5–1.0 mg/L	The final concentration of iron in ID-CAMHB prepared using this method should be ≤ 0.03 mg/L. Refer to CLSI M07[1] for calculating the amount of Ca^{2+}, Mg^{2+}, and the table below for calculating the amount of Zn^{2+} needed.

If CAMHB is being used at this step, it is not necessary to adjust the cations in the media until Step 7 as they will be chelated in Step 3.

If new to the preparation of ID-CAMHB or if a new reagent or manufacturer is being used for preparation, it is recommended that testing for residual iron should be conducted...

8	Check the pH to determine whether it is 7.3 ± 0.1.	If the pH exceeds 7.4, adjust it using 1 or 6 N HCl (use of 6 N HCl will minimize the volume required to adjust the pH). If the pH is below 7.2, use 2.5 N NaOH.
9	Filter the final product using a 0.2-µm filter.	
10	Store the media at 4 to 8°C for up to 2 mo.	

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; h, hour(s); HCl, hydrochloric acid; ID-CAMHB, iron-depleted cation-adjusted Mueller-Hinton broth; mo, month(s); NaOH, sodium hydroxide; pH, negative logarithm of hydrogen ion concentration.

Example for adding Zn^{2+} back to cation-adjusted Mueller-Hinton broth that contains below-detectable concentrations (< 0.0001 mg/L) of Zn^{2+} after chelation in step 3[2]:

Step	Action	Comments
1	Calculate the amount of Zn^{2+} needed using this formula: Final amount needed − amount in medium = amount to be added	For Zn^{2+}, the final amount needed is 0.5–1 mg/L. 1 mg/L − 0 mg/L = 1 mg/L
2	Add 1.54 mL Zn^{2+} stock per L (1.54 mL for each 1 mg/L).	C = concentration, V = volume $C_1 \cdot V_1$ = desired $C_2 \cdot$ final V_2 0.65 mg/mL $Zn^{2+} \cdot V_1$ = 1 mg Zn^{2+} /1000 mL · 1000 mL V_1 = 1 mg ÷ 0.65 mg/mL V_1 = 1.54 mL of Zn^{2+} stock
3	Proceed with steps 8 and 9 above.	

Footnote

a. Ensure all reagents (eg, deionized water to prepare acid and base and cation solutions) have been verified as having an iron content of ≤ 0.03 mg/L.

Step 9
It is recommended that testing for residual iron should always be conducted after media preparation is complete to confirm iron content does not exceed 0.03 mg/L. See step 5 for further guidance.

Step 10
Caution should be taken not to reintroduce cations during inoculum preparation. ID-CAHMB may be used for inoculum preparation to prevent the reintroduction of cations.

- Methods Working Group Discussion and Recommendation
  - Clarifications to methods
  - Use of MHB or CAMHB – If laboratory already has CAMHB, use that; if not use MHB
  - It is possible to saturate resin therefore start with MHB
  - Differences in media require different times of chelation
  - If iron is too high start with MHB
  - Clarify for CAMHB to not add cations before chelation.
  - Motion to accept changes as presented on both slides to include MHB or CAMHB. WG Vote: 8-0-1-3.
  - Motion to clarify not to add cations before chelation. Vote: 8-0-1-3.

SC DISCUSSION (MAIN POINTS)

- Step 10 states “cations” but the word should be “iron”. Should read as follows “Caution should be taken to not introduce iron during inoculum preparation. ID-CAMHB may be used for inoculum preparation to prevent the reintroduction of iron.”

A motion to accept the proposed edits and comments to Table H1.2 with modified language in step 10 to change "cation" to "iron" was made and seconded. Vote: 12 for, 0 against, 0 abstain, 2 absent (Pass)

CEFIDEROCOL AD HOC WORKING GROUP REPORT CONTINUED – QUALITY CONTROL

- QC Strain Recommendations
  - Evaluated additional isolates as quality control for media iron content
    - Several strains were identified that showed reproducible discrepant MIC values between ID-CAMHB and CAMHB prepared from different media sources
    - No isolate could be identified that showed discrepant MIC values between ID-CAMHB and CAMHB across all sources of media
    - Alternative methods to quality control iron media content would have to be pursued
  - AHWG Proposal: *P. aeruginosa* SR27001 (Shionogi isolate)
    - If elevated MIC (> 4 µg/ml; need to define cutoff) – repeat preparation of media
    - Akin to thymidine content QC for TMP-SMX
    - Supplemental QC
    - Shionogi will look into the process of depositing to ATCC and NCTC, NCNB
- Methods Working Group agreed for AHWG to move forward with this strain and present data at the June 2025 meeting

SC DISCUSSION (MAIN POINTS)

- CLSI has well outlined Tier 2 studies, what CLSI does not have is a focused less intensive study for things that are used for media QC that would be done by the manufacturer.
- Need to be specific on why and when this is needed, like for *E. faecalis* 29212 with trimethoprim-sulfamethoxazole for thymidine. Be clear that routine laboratories do not need to test this, it is for manufacturers.
- Should define what is sufficient QC to do that. Probably do not need the laboratory-to-laboratory variability in this specific case.
- Just like CLSI mentions for *Enterococcus faecalis* and trimethoprim-sulfamethoxazole that an MIC above a specific value indicates that laboratories should consider a problem with thymidine content in the media, something similar could be done here for iron and cefiderocol. The data needed to do this has already been generated by Shionogi and needs to be organized and presented to answer this question. This data should be brought to the June meeting.

CEFIDEROCOL AD HOC WORKING GROUP REPORT CONTINUED – DISK DIFFUSION

- Background
  - Disk diffusion testing of cefiderocol is complex
    - Appearance of (micro)colonies within zone of inhibition
    - Different Mueller-Hinton agar sources can produce different inhibition zone sizes
    - Poor correlation of small zone of inhibition (<15 mm) with broth microdilution MIC values for *A. baumannii*
  - Shionogi conducted studies to address these issues
    - Can colonies within zone of inhibition be ignored to facilitate zone read-out and enhance reproducibility?
      - Would outer zones provide better correlation with broth microdilution MIC values for *A. baumannii*?
      - Would outer zones provide better correlation with *in vivo* efficacy?
    - Do certain sources of Mueller-Hinton agar provide a better correlation with broth microdilution MIC values?

- Are certain sources of Mueller-Hinton agar more predictive of efficacy in a mouse thigh infection model?

- Methodology
  - Zone diameter determinations by disk diffusion
    - Two different brands of disk: MAST/Hardy and Liofilchem
    - Two different brands (one lot for each brand) of Mueller-Hinton Agar: BD BBL and BioMérieux (pre-made)
    - Perform testing over three days (three separate inocula)
    - Three replicates per isolate per media (same inoculum)
    - Total of 18 readings for each disk (36 total per strain)
  - Isolates tested for which *in vivo* and *in vitro* data are available
    - 7 *E. coli*, 17 *K. pneumoniae*, 14 *P. aeruginosa* and 47 *A. baumannii*
    - Cefiderocol MIC ranges from ≤0.06 - >32 µg/mL
  - Inner and outer disk zones were determined for *A. baumannii*
  - Bacterial inoculum (0.5 McFarland) controlled by nephelometer
    - Same inoculum was also used for broth microdilution
  - Assessed reproducibility of zone diameters across disks and media
  - Assessed categorical agreement with broth microdilution and *in vivo* efficacy
- Disk Diffusion Testing Using Mueller-Hinton Agar - Why not use ID-MHA?
  - Agar sequesters iron, creating an environment for cells to overexpress iron uptake systems
  - No need for iron-depleted Mueller Hinton agar for cefiderocol disk diffusion testing
- Appearance of Colonies Within Zone of Inhibition - Ignore or not
  - Some isolates show colonies within zone of inhibition
    - Mainly *A. baumannii* with elevated MIC values (≥1 µg/mL)
  - Complicates determination of zone of inhibition
  - Colonies within zone of inhibition should not be ignored
    - Some are resistant, others are not - depending on isolate
  - Outer zone should not be used
    - Poorer categorical agreement with MIC compared to inner zone
    - Poorer correlation with *in vivo* outcome compared to inner zone
  - Appearance of colonies within zone of inhibition depends on agar
  - Growth within inner zones should not be ignored based on categorical agreement
    - Inner zones provide better categorical agreement with MIC values
  - Growth within inner zones should not be ignored based on *in vivo* efficacy
    - When reading outer inhibition zones, several isolates test as susceptible, but show an increase in bacterial load in mouse thigh infection model
    - Inner zones do not produce such errors
    - Inner zones correlate better with *in vivo* efficacy
- Reproducibility of Zone of Inhibition Measurements
  - Over 90% of the zone of inhibition measurements were within ±2 mm of modal zone of inhibition for each media/disk combination for Enterobacterales

- Over 88% of the zone of inhibition measurements were within ±2 mm of modal zone of inhibition for each media/disk combination for *P. aeruginosa*
- Over 90% of the outer zone of inhibition measurements were within ±2 mm of modal zone of inhibition for each media/disk combination for *A. baumannii*
- For the inner zone of inhibitions, over 90% of measurements were within ±2 mm of modal zone of inhibition for BD media, but reproducibility was less for bioMérieux media (~85%)
  - Attributable to less frequent appearance of (micro)colonies within zone of inhibition on bioMérieux agar

- Disk Diffusion Testing-Media and Disk
  - Disks from different manufacturers show similar zones of inhibition, but different media show slightly different zones of inhibition
  - BD shows smaller zones of inhibition for *P. aeruginosa* and *A. baumannii*, but not for Enterobacterales
- MIC (BD-BBL Medium) and Zone of Inhibition Correlation
  - No pattern observed for errors with media or disks
  - Minor errors and two major errors observed with Enterobacterales and *P. aeruginosa*
    - Disks under-report susceptibility (over-report intermediate/resistance)
  - Errors will differ if compared to MIC values obtained with different Mueller-Hinton broths
- *In vivo* Efficacy and Zone of Inhibition Correlation
  - Zones of inhibition indicating susceptibility were predictive of efficacy in animal model
- Conclusions
  - Zone of inhibition measurements are reproducible
    - Reproducibility is affected by the appearance of (micro)colonies within zone of inhibition
  - Colonies within zone of inhibitions should not be ignored
  - Disks from Mast/Hardy and Liofilchem produce equivalent inhibition zones
  - Media affects size of inhibition zone, but no big impact on susceptibility/intermediate/resistance calls
    - Very little cross over breakpoints
  - When compared to MIC values obtained in BD-BBL broth, zones of inhibition under report susceptibility
    - Under-reporting less frequent when compared to MIC values obtained with Oxoid or Difco broth
  - Zone of inhibition susceptibility correlated well with *in vivo* efficacy
  - Despite limitations, disk diffusion remains a reliable method to assess susceptibility for cefiderocol
  - No changes in reading guidelines for zone of inhibition when using disk diffusion are recommended
- EUCAST Disk and Media Evaluation Study
  - EUCAST breakpoints were established using disks from Liofilchem and Mast and MH agar from BBL and Oxoid
  - Data corelated but some laboratories were having issues
  - Evaluation of disks and media from several manufacturers
    - Cefiderocol 30 µg disks from Liofilchem, Mast and Oxoid.
    - MH agar from
      - BBL, Bio-Rad and Oxoid (in-house produced plates)
      - bioMérieux and Liofilchem (prepared plates)
  - EUCAST Warning
    - https://www.eucast.org/ast-of-bacteria/warnings

- If mean values of at least 5 repeated tests for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 are within ±1 mm of the target values, disk diffusion using EUCAST breakpoints performed well.
- When the mean value was more than ±1 mm from the target, an increasing proportion of results were erroneous. This was particularly problematic for *Pseudomonas aeruginosa*.
- Among the evaluated cefiderocol 30 µg disks (Liofilchem, Mast and Oxoid) and Mueller-Hinton agars (BBL, bioMérieux, Bio-Rad, Liofilchem and Oxoid), disks from Oxoid and MH agar from Bio-Rad produced larger than acceptable zone diameters for both QC strains and clinical isolates. Combining Oxoid disks with Bio-Rad MH agar increased the problem further.

- Methods Working Group decided next step is for the ad hoc working group to discuss target values and modes.

SC DISCUSSION (MAIN POINTS)

- If the QC is on the higher range, then start to see categorical errors with clinical isolates. AHWG would like to meet with the QC Working Group to set target modes for QC to control this issue.
- The wild-type and non-wild-type are overlapping for disk diffusion distribution.
- Colonies within the zone size of disk diffusion are not reproducible.
- Concern that *Acinetobacter* disk diffusion does not work. Is the Subcommittee comfortable leaving the disk diffusion breakpoints?
  - CLSI should look at clinical failure signals.
  - In June, can use the QC mode to determine if disk diffusion works for *Acinetobacter* or if CLSI needs to remove disk diffusion.
  - CLSI probably needs to re-assess the cefiderocol breakpoints for *Acinetobacter* at a future meeting.
- EUCAST thinks their data looks acceptable. EUCAST looked at different disk potencies and will likely go back and see if they can find a better disk. They do see some media related variability studies.
- With a susceptible MIC breakpoint of 4 µg/mL, the overlap becomes even more problematic.
  - If the breakpoint is changed to an MIC to 1µg/mL, it would make some problems go away.
- There have been similar challenges with inner colonies and fosfomycin. Does CLSI need better guidance in the CLSI M100 about inner colonies? Note EUCAST and CLSI treat inner colonies differently.
  - *Pseudomonas* inner colonies are known to have mutations.
  - Fosfomycin inner colonies are usually seen with other organisms, but only officially test *E. coli* and the inner colonies do have mutations for *E. coli* when they do occur.
  - CLSI needs to re-emphasize the inner colonies are read, which is CLSI's general policy. CLSI could add in reading guides with images for disk diffusion.
- A disk with inner colonies is then reflexed to BMD. There was concern a susceptible BMD result is not trustable. BMD is not specifically designed to look for heteroresistance.
- One laboratory commented that if they see a zone size less than 15 mm with inner colonies for *Acinetobacter*, they avoid using cefiderocol and use sulbactam-durlobactam instead.
- Send data on *Acinetobacter* and cefiderocol to Trish Simner.

3. **TEXT AND TABLE WORKING GROUP (A. BOBENCHIK)**

ORAL CEPHALOSPORINS

- Questions (emailed 10/29/24):
  - Unclear if oral cefuroxime breakpoints apply only to the 3 species of Enterobacterales indicated in comment (21) or do oral cefuroxime breakpoints apply to all Enterobacterales?
  - Do oral cefuroxime breakpoints only apply to uUTIs (ie, referring back to comment (21)) or is there no site restriction?
- "See comment (21)." also listed for: loracarbef, cefaclor, cefdinir, cefpodoxime, cefprozil

Table 2A-1. Enterobacterales (excluding *Salmonella/Shigella*) (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
CEPHEMS (ORAL)										
Cefazolin (U)[b] (surrogate test for oral cephalosporins and uncomplicated UTIs)	30 µg	≥ 15	–	–	≤ 14	≤ 16	–	–	≥ 32	**(21)** Breakpoints are for cefazolin when used as a surrogate test to predict results for the oral agents cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime, cephalexin, and loracarbef when used for therapy of uncomplicated UTIs due to *E. coli*, *K. pneumoniae*, and *P. mirabilis*. Cefazolin tested as a surrogate may overcall resistance to cefdinir, cefpodoxime, and cefuroxime. If cefazolin tests resistant, test these drugs individually if needed for therapy.
Cefuroxime (oral)	30 µg	≥ 23	–	15–22^	≤ 14	≤ 4		8–16^	≥ 32	See comment (21).

- Species other than *E. coli*, *K. pneumoniae*, and *P. mirabilis* in CLSI M100 Appendix B shown without intrinsic resistance for cefuroxime

B1. Enterobacterales

Organism ↓ / Antimicrobial Agent →	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporins II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline	Nitrofurantoin	Polymyxin B Colistin	Aminoglycosides
Citrobacter freundii	R	R	R		R	R	R						
Citrobacter koseri, Citrobacter amalonaticus group[a]	R			R									
Enterobacter cloacae complex[b]	R	R	R		R	R							
Escherichia coli	There is no intrinsic resistance to β-lactams in this organism.												
Escherichia hermannii	R			R									
Hafnia alvei	R	R	R		R	R						R[c]	
Klebsiella (formerly *Enterobacter*) *aerogenes*	R	R	R		R	R							
Klebsiella pneumoniae, Klebsiella oxytoca, Klebsiella variicola	R			R									
Morganella morganii	R	R			R		R	[d]		R	R	R	
Proteus mirabilis	There is no intrinsic resistance to penicillins and cephalosporins in this organism.							[d]	R	R	R	R	
Proteus penneri	R				R		R	[d]	R	R	R	R	

B1. Enterobacterales (Continued)

Organism ↓ / Antimicrobial Agent →	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporins II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline	Nitrofurantoin	Polymyxin B Colistin	Aminoglycosides
Proteus vulgaris	R				R		R	[d]	R	R	R	R	
Providencia rettgeri	R	R			R			[d]	R	R	R	R	
Providencia stuartii	R	R			R			[d]	R	R	R	R	[e]
Raoultella spp.[f]	R			R									
Salmonella and *Shigella* spp.	There is no intrinsic resistance to β-lactams in these organisms; refer to **WARNING** below for reporting.												
Serratia marcescens	R	R	R		R	R	R				R	R	
Yersinia enterocolitica	R	R		R	R								

- FDA label for oral cefuroxime

CEFTIN (cefuroxime axetil) tablets, for oral use
CEFTIN (cefuroxime axetil), for oral suspension
Initial U.S. Approval: 1987

----------INDICATIONS AND USAGE----------

CEFTIN is a cephalosporin antibacterial drug indicated for the treatment of the following infections due to susceptible bacteria: (1)

- Pharyngitis/tonsillitis (adults and pediatric patients) (1.1)
- Acute bacterial otitis media (pediatric patients) (1.2)
- Acute bacterial maxillary sinusitis (adults and pediatric patients) (1.3)
- Acute bacterial exacerbations of chronic bronchitis and secondary bacterial infections of acute bronchitis (adults and pediatric patients 13 years and older) (1.4)
- Uncomplicated skin and skin-structure infections (adults and pediatric patients 13 years and older) (1.5)
- Uncomplicated urinary tract infections (adults and pediatric patients 13 years and older) (1.6)
- Uncomplicated gonorrhea (adults and pediatric patients 13 years and older) (1.7)
- Early Lyme disease (adults and pediatric patients 13 years and older) (1.8)
- Impetigo (pediatric patients) (1.9)

Cefuroxime axetil has been shown to be active against most isolates of the following bacteria, both in vitro and in clinical infections *[see Indications and Usage (1)]*:

- Gram-negative bacteria
  Escherichia coli[a]
  Klebsiella pneumoniae[a]
  Haemophilus influenzae
  Haemophilus parainfluenzae
  Moraxella catarrhalis
  Neisseria gonorrhoeae

The following in vitro data are available, but their clinical significance is unknown. At least 90 percent of the following microorganisms exhibit an in vitro minimum inhibitory concentration (MIC) less than or equal to the susceptible breakpoint for cefuroxime axetil of 1 mcg/mL. However, the efficacy of cefuroxime axetil in treating clinical infections due to these microorganisms has not been established in adequate and well-controlled clinical trials.

- Gram-negative bacteria
  Morganella morganii
  Proteus inconstans
  Proteus mirabilis
  Providencia rettgeri

- Text and Tables Working Group Discussion and Recommendation
  - FDA package insert says it applies only to *K. pneumoniae*, also only pertains to urinary tract infection
  - The list of oral cephalosporins is not clear and EUCAST does not list these. This should be confined to *K. pneumoniae*.
  - Comment 21 seems fine, but the cefuroxime breakpoints should not have the comment 21 reference
  - Overall, confusion around how to apply the oral cephalosporin breakpoints and if there needs to be additional notation/clarification
  - For those that refer to comment (21), are they only indicated for the *E. coli, K. pneumoniae*, and *P. mirabilis* from uUTI only?
    - If yes, then would clarify this with a (U) designation and call this out in a comment for each
    - If no, then would need additional clarification that it is not restricted for *K. pneumoniae* and uUTIs

SC DISCUSSION (MAIN POINTS)

- USCAST has some data that CLSI should review and discuss in the future.
- Laboratories do not know if a patient has a complicated UTI.
- It is good to limit cefuroxime to just the three organisms: *E. coli, K. pneumoniae,* and *P. mirabilis*.
- This is a breakpoints issue.
- Consider the pediatric perspective.
- The “see comment 21” seem like it is part of the confusion.

- How to report this? What about 3rd generation cephalosporins?
- This sounds like a project for Breakpoints Working Group.
- Drug package inserts say to use cephalothin to predict susceptibility, but CLSI changed this to use cefazolin as the predictor.

A motion to remove the "see comment 21" in the comments column for cefuroxime, loracarbef, cefaclor, cefdinir, cefpodoxime, and cefprozil in Table 2A-1 was made and seconded. Vote: 12 for, 0 against, 0 abstain, 2 absent (Pass)

BREAKPOINTS ADDITIONS/REVISIONS CLSI M100 TABLES

- Separate out *Salmonella/Shigella* from Enterobacterales to align with newer split into Tables 1A-2/2A-2
- Split out and update name for Enterobacterales section in the CLSI Breakpoints Additions Since 2010 Table
    - Note: leave in Enterobacterales information but removing "Non-*Salmonella* spp." detail as it is no longer necessary once these are separated out

***SALMONELLA/SHIGELLA* TABLE 2A-2**

- During discussion of comment harmonization between Table 1A-2 and new Table 2A-2, a comment is needed about how to address drugs that were not brought over into Table 2A-2
- For example:
    - Questions from users on whether cefepime and piperacillin-tazobactam Table 2A-1 Enterobacterales breakpoints could still be used
    - Manufacturer commented that updates were made to expert rules to suppress reporting of all drugs not represented in Table 2A-2 for *Salmonella/Shigella* isolates. Previously, they would be reported as they were included in the old Table 2A when these organisms were combined with Enterobacterales.
    - Some examples below:
        - aztreonam
        - β-lactamase inhibitor combinations (including piperacillin-tazobactam)
        - colistin, polymyxin B
        - doripenem
        - folate pathway antagonists
        - etc.
    - Issues to address:
        - Should there be any guidance for users on what to do if drugs are not listed in the table?
        - Should other drugs from Table 2A-1 be considered for Table 2A-2?

SC DISCUSSION (MAIN POINTS)

- Need to check with CDC for data.
- CLSI thinks these drugs were pulled out intentionally but need to go back and review.
- Physicians are asking for carbapenems, but there is a question about if these drugs get into intracellular organisms. There is maybe a little data in ertapenem. There are XDR isolates, where carbapenems might be the only option.
- Consider adding a note that specific drugs are not for *Salmonella*.
- A full AHWG is not needed.

- Action item: Review the previous data and discussions to clarify the details here.

DEFFERRED COMMENT FROM REVIEW

- Table 1J Anaerobe footnote c, should this be attached to penicillin (gram-positive anaerobes) in Tier 1 or only to penicillin (gram-negative anaerobes) in Tier 4?

Table 1J. Anaerobes

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ampicillin (gram-positive anaerobes)[a,b] Penicillin (gram-positive anaerobes)[a,b,c]			Ampicillin (gram-negative anaerobes)[a,b] Penicillin (gram-negative anaerobes)[a,b,c]
Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam			

c. Penicillin retains good *in vitro* activity against most Fusobacterium spp. and may be considered for primary testing and reporting with this genus. **β-Lactamases have been reported in this genus.**

- Text and Tables Working Group agreed to remove footnote c from Tier 1 penicillin (gram-positive anaerobes)

5. **OUTREACH WORKING GROUP (J. HINDLER)**

WORKING GROUP GOALS

- Educate practicing clinical microbiologists and health care professionals about AST practices and recommendations.
- Provide resources to facilitate individuals in their understanding and implementation of CLSI AST recommendations.
- Solicit suggestions from members of other CLSI Working Groups for educational activities; encourage AST Subcommittee volunteers to engage in these educational activities.
- Note: it is beyond the purview of Outreach Working Group to interpret data or provide technical recommendations that may be highly controversial, inconsistent with current or prior AST Subcommittee decisions, or that have not been confirmed by the AST Subcommittee.

PRODUCTS OF WORKING GROUP

- Education Workshops
- News updates
- Webinars
    - CLSI/Society of Infectious Diseases Pharmacists (SIDP)/American College of Clinical Pharmacy (ACCP)
    - CLSI/College of American Pathologists (CAP)
    - Other
- Programs at other meetings (eg, ASM, IDWeek)
- Other educational products
    - CLSI M100 Educational Program (2024 updates in progress)
    - Breakpoint Implementation Toolkit (BIT) and accompanying materials
- Other publications
    - Annual mini review of new CLSI M100
    - Other

CLSI AST SUBCOMMITTEE - EDUCATION NEEDS NOW

- Highest Priority
    - New QC recommendations
    - *Burkholderia cepacia* – lack of breakpoints, use of epidemiologic cutoff values (ECVs)
- Other
    - Performing and reporting AST on *Candida auris*
    - Testing options with/without commercial ASTs for non-cleared species
    - Breakpoint updates
    - Impact of breakpoint changes on surveillance data and cumulative antibiograms

WEBINARS/PRESENTATIONS

- CLSI-SIDP-ACCP Annual Webinar
    - Breaking Bad Bacteria: Mastering the 2024 CLSI Antimicrobial Susceptibility Testing Updates
    - August 7, 2024

- Speakers: Virginia Pierce and Navaneeth Narayanan
- August 2024 stats:
  - 337 CLSI members registered (50 for 2023 CLSI/SIDP/ACCP annual webinar)
  - 569 joined the live webinar (519 for 2023 CLSI/SIDP/ACCP annual webinar)
  - 279 on-demand views from CLSI members

- CLSI-CAP Annual Webinar
  - Identification and Methicillin Resistance Testing for Staphylococci Other than S. *aureus* (SOSA) – What to do now?
  - December 5, 2024
  - Speakers: Jennifer Dien Bard and Lars Westblade
  - January 2025 stats:
    - 102 registered
    - 93 joined the live webinar
    - 27 on-demand views as of end of 2024
- CLSI Annual Update (22nd)
  - What's New in the 2025 CLSI Standards for Antimicrobial Susceptibility Testing (AST)?
  - February 26, 2025
  - Speakers: April Bobenchik and Romney Humphries
  - Moderator: Janet Hindler
- Webinar 2025
  - What should clinical laboratories know about LDT regulations as related to AST?
  - Date TBD
  - Format:
    - Panel Discussion (clinical and public health laboratories)
    - Moderator – pose questions
    - Up to 5 laboratory directors from various settings answer how their laboratory is addressing this

ASM MICROBE 2024

- CLSI Updates of New ß-lactam Combination Agents and Other Novel Antimicrobials
  - Lounge and Learn
  - Saturday, June 22, 2025
  - 10:45 AM PST
  - Speaker: Romney Humphries
  - Moderator: Priyanka Uprety

ATTENDEE ORIENTATION

- Updated for June 2024
- On demand via YouTube as CLSI New Member Orientation

BREAKPOINT IMPLEMENTATION TOOLKIT (BIT)

- Launched June 2023
- Updated in June 2024

CLSI M100 EDUCATIONAL PROGRAM

- No fee
- Enhance user ease of access
- Great for laboratory directors, training technologists and other trainees in laboratory
- CLSI M100 34th edition released November 2024
    - New look
    - Audrey Schuetz narrating!
    - Sections:
        - Using CLSI M100
        - Exercises
    - Remove setting disk breakpoints to separate section
- Updating to CLSI M100 35th edition
- 392 new registrations in 2024
- 177 learners accessed the course

CLSI M02 /M07 EDUCATIONAL PROGRAM

- Based on CDC Antimicrobial Susceptibility Testing Training "Master" CD ROM from 2002
    - Subsequently on CDC's website
    - Removed 2019 due to lack of resources to maintain the program
- Interactive
- Reflect "how to" for bench techs

ORWG NEWS UPDATE

- Publication goals: March, September
- Fall 2024 (delayed)
    - Feature: *Stenotrophomonas maltophilia*
    - Case: Reporting cefepime for carbapenemase producers
    - Practical tips: Linezolid/tedizolid
    - Hot topic: New antifungal drug rezafungin
- Spring 2025
    - Feature: *Burkholderia cepacia*
    - Case: *Candida auris* AST
    - Practical tips: QC – new recommendations
    - Hot topics
        - FDA breakpoint developments
        - Anaerobe Working Group AST status and CLSI M11

- Recent developments
  - Disk diffusion not a reference method
  - Voriconazole *A. fumigatus* breakpoint recognized by FDA, other new breakpoints from Antifungal AST Subcommittee
  - CLSI M100 Educational Program (how access)

AST SC MEETING EDUCATION SESSIONS

- January 2025
  - Strategies for Addressing Three Noteworthy Antimicrobial Resistance Challenges
  - Speakers: Kevin Alby, Nathan Wiederhold, and Stephen Cole
  - Moderator: Stella Antonara
  - Will be available for on-demand viewing and 1.5 PACE credit!
- June 2025
  - Where Are We Now With Whole Genome Sequencing?
  - Clinical, pharmaceutical, public health

PUBLICATIONS

- Schuetz, A, A Ferrell, J Hindler, R Humphries, A Bobenchik. Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100 32nd and 33rd Editions. JCM. In Press.
- Bobenchik, A, A Ferrell, J Hindler, A Schuetz. Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100 34th Edition.

QUALITY CONTROL

- Annual CLSI M100 Webinar
- Rationale document, implementation tools (including short video)
- Scenario examples, eg,
  - "Old drugs on panel in use for 4 years"
  - Newer disk (eg, cefiderocol) introduced 3 months ago
- Revisit practical approaches to ensuring reliable AST results

BURKHOLDERIA CEPACIA

- Guidance document from the *Burkholderia cepacia* complex AHWG
- *Burkholderia cepacia* FAQs for clinical laboratories

SC DISCUSSION (MAIN POINTS)

- There is concern about using the ECV for *Burkholderia*, so might not want to publicize that information too much.
  - The ECV AHWG is working with the *B. cepacia* complex AHWG. The trimethoprim-sulfamethoxazole ECV is troublesome.
- CLSI needs to vote on the *Burkholderia cepacia* guidance document if CLSI will post it on their website.
- Where can laboratories send testing for *Burkholderia*?
  - LabCorp, ARUP, Mayo Clinic, and Quest do not currently offer reference method BMD for *Burkholderia*.

- LSI was asked if they could offer reference method BMD, and LSI said they could possibly consider it.
- It is not just about AST accuracy, there was concern that some drugs might not be active because of PK/PD data.
- Dry form MIC panels were not evaluated, so it might perform equivalent to the reference method
  - AHWG did actually look at dry lyophilized commercial panels and the error rates are too high.
  - Was this data generated side-by-side with the same inoculum? Is this published?
  - This was compared to the data set generated from the AHWG with modal MICs with reference testing and they are repeating the testing now to confirm results. A few drugs look OK, but in general they drugs do not meet 90% agreement.
- Did CLSI talk to the cystic fibrosis (CF) foundation?
  - Yes, CLSI did. There is a Practical Guidance in Clinical Microbiology document in Clinical Microbiology Reviews written in part by the CF foundation on this topic (https://journals.asm.org/doi/10.1128/cmr.00215-21).
  - Perhaps more people at the CF foundation need to be included in the discussions.
- Perhaps public health could offer testing. Does the Michigan laboratory do any testing?
  - Not present to comment.
- LSI is set up to do new individual new agents, but maybe they could consider offering this.
- There is a lack of access to reference method BMD, this is the tip of the iceberg, there is a larger problem. Should CLSI continue to make this guidance of reference BMD if there is no way for laboratories to gain access to that testing?
- Remember that the whole reason the breakpoints were removed was because the Subcommittee was not confident that the AST data matched the clinical experience.

VOLUNTEER OPPORTUNITIES

- News Update
  - Provide feedback on content, delivery, and structure
  - Suggest content
  - Partner with others to write articles (case studies and more)
- Other Publications
  - Assorted topics
- Webinars / Workshops / Lectures
  - Suggest content
  - Speakers
- Other Projects
- If anyone is asked to talk about CLSI, please coordinate with Outreach Working Group.

6. **M45 WORKING GROUP (R. HUMPHRIES)**

TIMELINE FOR CLSI M45

- Final Draft to Working Group: January 20, 2025
- Second Round of Working Group Comments: February 14, 2025
- CLSI Preparation and Editing: February 2025- April 2025
- Proposed Draft Vote and Comment (public review): April 2025 – May 2025
- Proposed Draft Comment Resolutions: May 2025 – July 2025
- CLSI Final Draft Editing: August 2025 – November 2025
- Final Draft Vote and Comment (Consensus Council review): December 2025 – January 2026
- Publication: March 2026 – April 2026

EXCITING DEVELOPMENT

- FDA recognition of majority of CLSI M45 breakpoints (January 15, 2025)
- Exceptions to FDA M45 recognition
    - Chloramphenicol (*Abiotrophia/Granulicatella*, *Aeromonas*)
    - Clindamycin (*M. catarrhalis*)
    - Rifampin (HACEK and *M. catarrhalis*)
    - **All other breakpoints are recognized by FDA!**

MAJOR UPDATES TO M45

- New tables
    - *Achromobacter* spp.
    - Non-aeruginosa *Pseudomonas*
    - *Capnocytophaga* (ß-lactamase test only)
- Expanded organism groups

M45 3rd Ed	M45 4th Ed
Bacillus and... *Brevibacillus, Cohnella, Lysinibacillus, Paenibacillus, Sporolactobacillus*	***Bacillus and ...*** *Brevibacillus,* ***Cytobacillus****, Lysinibacillus,* ***Neobacillus, Niallia****, Paenibacillus,* ***Peribacillus, Priestia, Robertmurraya, Shouchella, Sutcliffiella****, and* ***Weizmannia***
Campylobacter jejuni / coli	*Campylobacter jejuni / coli* ***C. upsaliensis, C. lari, C. fetus,*** **and** ***C. doylei*** (MIC only)
Corynebacterium and related genera (including R. dentocariosa)	*Related genera does not include R. dentocariosa*

M45 3rd Ed	M45 4th Ed
Gemella	*Gemella* & **other catalase negative GPC (*Facklamia* spp., *Dolosigranulum* spp. and *Globicatella* spp but not *Ignavigranum spp.)**
Lactobacillus spp.	*Lactobacillus* **and 25 other genera (2020 reclassification)**
Leuconostoc spp.	*Leuconostoc and Weisella**
Moraxella catarrhalis	*Moraxella* spp.
Bacillus anthracis	Bacillus anthracis Bacillus cereus biovar anthracis

- New MIC breakpoints
  - *Abiotrophia* and *Granulicatella* spp., linezolid
  - *Aerococcus* spp., ampicillin, nitrofurantoin
  - *Bacillus* spp. doxycycline and linezolid
  - *Campylobacter*, meropenem, imipenem and azithromycin
  - *Gemella* spp., daptomycin, linezolid and doxycycline
  - *H. pylori*, amoxicillin and levofloxacin
  - *Leuconostoc*, daptomycin and clindamycin
  - *Micrococcus*, trimethoprim-sulfa, doxycycline and daptomycin
  - *Moraxella*, meropenem, Lefamulin
  - *Pediococcus*, daptomycin, linezolid, levofloxacin, clindamycin
  - *Rothia*, doxycycline
  - *Vibrio*, azithromycin
- Major breakpoint revisions
  - Penicillin and ampicillin (based on "best estimate" PK data & ECV)
    - *Abiotrophia and Granulicatella*
    - *Aerococcus*
    - *Bacillus* (removed)
    - *Corynebacterium*
    - *Lactobacillus*
    - *Lactococcus*
    - *Leuconostoc*
    - *Micrococcus*
    - *Pediococcus*

- Tetracyclines
  - *Aeromonas*
  - *Campylobacter*
  - *Corynebacterium*
  - *HACEK*
  - *Lactococcus*
  - *Leuconostoc*
  - *Vibrio*
- Breakpoint updates – alignment with CLSI M100
  - *Aeromonas* and *Vibrio* with Enterobacterales
    - Piperacillin-tazobactam
    - Cefepime
    - Aminoglycosides
    - Fluoroquinolones
- New intrinsic resistance tables

BREAKPOINTS THAT ARE "RETIRED"

- *Aerococcus*
  - Trimethoprim-sulfamethoxazole
- *Bacillus* spp.
  - Ampicillin, penicillin
  - Amikacin, gentamicin
- *Corynebacterium* spp.
  - Cefepime, cefotaxime, ceftriaxone, imipenem
  - Teicoplanin, daptomycin, quinupristin-dalfopristin
  - Erythromycin, clindamycin
  - Chloramphenicol, rifampin
- *Erysipelothrix rhusiopathiae*
  - Imipenem, gatifloxacin
- *Gemella*
  - Erythromycin, clindamycin
- *Aggregatibacter*
  - Imipenem, meropenem
- *Lactobacillus*
  - Gentamicin

ADDITIONAL WORK

- Added table of "changes" to breakpoints with dates
- Refer most QC to CLSI M100

	• Updated definitions to align with CLSI M02, M07, M100
	• Updated resistance sections for all Tables
	• Updated references
	• Addressed errors identified in the M45 3rd Ed
	
	SC DISCUSSION (MAIN POINTS)
	• Dosages are listed in CLSI M100, the M45 points users to the CLSI M100 for dosages.
	• The FDA did not accept the CLSI breakpoints for the following combinations. Is there a need for these breakpoints?
	  ○ Clindamycin and *Moraxella*
	  ○ Rifampin for HACEK and *M. catarrhalis*
	• Are commercial companies going to update the package inserts?
	  ○ The commercial companies will have to look on a case-by-case basis. It might not be worth the resources of commercial companies.
	• Did the M45 Working Group look at *Pseudomonas* non-*aeruginosa* organisms for carbapenems?
	  ○ There is mCIM and WGS data, but the working group has not truly accessed this.
	• There was a request to have a newsletter or rationale document on removing daptomycin MICs and *Corynebacterium*.

7.	**ADJOURNMENT** Dr. Mathers thanked the participants for their attention. The meeting was adjourned at 12:00 PM Eastern Standard (US) time.

PLENARY ATTENDEES

Plenary 1	Plenary 2	Plenary 3
Adams Jennifer K.	Adams Jennifer K.	Adams Jennifer K.
Alby Kevin	Alby Kevin	Alby Kevin
Ambler Jane E.	Ambler Jane E.	Antonara Stella
Antonara Stella	Antonara Stella	Arbefeville Sophie
Arbefeville Sophie	Arbefeville Sophie	Asempa Tomefa
Asempa Tomefa	Asempa Tomefa	Austerman Ashley
Atkinson Dunn Robyn	Austerman Ashley	Bala Shukal
Austerman Ashley	Bala Shukal	Balbuena Rocio
Bala Shukal	Balbuena Rocio	Baptie Pennie
Balbuena Rocio	Baptie Pennie	Barber Meagan
Baptie Pennie	Barber Meagan	Barnett Katie
Barber Meagan	Barnett Katie	Belley Adam
Barnett Katie	beiner linette	Berkow Elizabeth
beiner linette	Belley Adam	Bhalodi Amira
Belley Adam	Bensman Timothy J.	Bhatnagar Amelia
Bensman Timothy J.	Berkeley Lynette Y.	Bhatti Micah M.
Berkeley Lynette Y.	Berkow Elizabeth	Bhavnani Sujata M.
Berkow Elizabeth	Bhalodi Amira	Bixby Morgan
Bhalodi Amira	Bhatnagar Amelia	Blosser Sara
Bhatnagar Amelia	Bhatti Micah M.	Bobenchik April M.
Bhatti Micah M.	Bhavnani Sujata M.	Boswell Malcolm
Bhavnani Sujata M.	Bixby Morgan	Bowden Robert
Bixby Morgan	Blosser Sara	Boyer Jennifer
Blosser Sara	Bobenchik April M.	Boyle Bridget
Bobenchik April M.	Boswell Malcolm	Bradford Patricia
Boswell Malcolm	Bowden Robert	Brandt Maryann
Bowden Robert	Boyer Jennifer	Brasso William B.
Boyer Jennifer	Boyle Bridget	Brown Carrine
Boyle Bridget	Bradford Patricia	Bryan, MD, PhD Andrew
Bradford Patricia	Brandt Maryann	Bryowsky Jason
Brandt Maryann	Brasso William B.	Bryson Alexandra Lynn
Brasso William B.	Brown Carrine	Bui-Bullock Tina
Brown Carrine	Bryan, MD, PhD Andrew	Bulman Zackery P.
Bryan, MD, PhD Andrew	Bryowsky Jason	Burbick Claire R.
Bryowsky Jason	Bryson Alexandra Lynn	Burgess David S
Bryson Alexandra Lynn	Bui-Bullock Tina	Burnham Carey-Ann
Bui-Bullock Tina	Bulman Zackery P.	Bush Karen
Bulman Zackery P.	Burbick Claire R.	Butler Deborah
Burbick Claire R.	Burgess David S	Caidi Hayat
Burgess David S	Burnham Carey-Ann	Campbell Davina
Burnham Carey-Ann	Bursens Jeroen	Campeau Shelley

Bursens Jeroen
Bush Karen
Bussian David
Butler Deborah
Caidi Hayat
Campbell Davina
Campeau Shelley
Capraro Gerald A.
Carpenter Darcie E.
Carvalhaes Cecilia
Castanheira Mariana
Chandler Courtney
Chandrasekaran Sukantha
CHEN YAMIN
Cintron Cotto Melvili
Cole Nicolynn
Copsey-Mawer Sarah
Craney Arryn
Cullen Sharon K.
Danielsen Zhixia
Debabov Dmitri
DeJonge Boudewijn
DeStefano Ian
Dial Courtney
Diaz-Campos Dubraska V.
Dien Bard Jennifer
Dingle Tanis
Donohue Lindsay
Dressel Dana C.
Dumm Rebekah
Duncan Elaine
Edelstein Paul
Eickhoff Michaela
Elanany Mervat
Esparza German
Ewald-Saldana Gina L.
Farley John
Fedorenko Marianna
Ferrell Andrea L.
Fisher Mark A.
Flemming Laurie
Forrest Graeme
Fratoni Andrew
Bush Karen
Bussian David
Butler Deborah
Caidi Hayat
Campbell Davina
Campeau Shelley
Capraro Gerald A.
Carpenter Darcie E.
Carvalhaes Cecilia
Castanheira Mariana
Castillo-Martinez Nydia
Chandler Courtney
Chandrasekaran Sukantha
CHEN YAMIN
Cintron Cotto Melvili
Cole Nicolynn
Copsey-Mawer Sarah
Craney Arryn
Cullen Sharon K.
Danielsen Zhixia
Debabov Dmitri
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Ewald-Saldana Gina L.
Farley John
Fedorenko Marianna
Ferrell Andrea L.
Fisher Mark A.
Flemming Laurie
Forrest Graeme
Fratoni Andrew
Gancarz Barb
Garg Rahul
Garner Cherilyn D.
Garrett Elizabeth
Gatermann Sören
Gefroh Sarah
Ghosh Mayurika
Giffen Samantha

Gancarz Barb
Garg Rahul
Garner Cherilyn D.
Garrett Elizabeth
Gatermann Sören
Gefroh Sarah
Ghosh Mayurika
Giffen Samantha
Gill Darcy
Gitman Melissa
Glaser Laurel
Glasgow Heather
Glover Janiece
Godwin Melissa
Goldstein Beth P.
Gomez Emily J.
Goodwin Avery
Grande Roche Kerian K.
Gray Alice
Gray Kamisha
Greninger Alex
Hackel Meredith
Haddock Christopher
Hamilton Lauren
Hendrix Megan
Hernandez Esther
Herrera Elide
Hill Brandon
Hindler Janet A.
Hirsch Elizabeth
Hoffard Rita
Holliday Nicole
Howell Nicholas
Hsiung Andre
Huband Michael D.
Humphries Romney M
Hunt Lauren
Huse Holly
Iarikov Dmitri
Jean Sophonie
Jimenez Pearson Antonieta
Johnson Brian
Johnson Kristie

Gancarz Barb
Garg Rahul
Garner Cherilyn D.
Garrett Elizabeth
Gatermann Sören
Gefroh Sarah
Ghosh Mayurika
Giffen Samantha
Gill Darcy
Gitman Melissa
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Gomez Emily J.
Goodwin Avery
Grande Roche Kerian K.
Gray Alice
Gray Kamisha
Greninger Alex
Hackel Meredith
Haddock Christopher
Hamilton Lauren
Hawser Stephen
Hernandez Esther
Herrera Elide
Hill Brandon
Hindler Janet A.
Hirsch Elizabeth
Hoffard Rita
Holliday Nicole
Howell Nicholas
Hsiung Andre
Huband Michael D.
Humphries Romney M
Hunt Lauren
Huse Holly
Iarikov Dmitri
Jean Sophonie
Jimenez Pearson Antonieta
Johnson Brian
Johnson Kristie
Jones Barb
Jung Sarah
Justo Julie Ann
Kahlmeter Gunnar
Kamau Edwin
Karlsson Asa
Kasapidis Cara
Kersh Ellen N.
Khalid Haziq

Jones Barb
Jung Sarah
Justo Julie Ann
Kahlmeter Gunnar
Kamau Edwin
Karlsson Asa
Kasapidis Cara
Kersh Ellen N.
Khalid Haziq
Khan Ayesha
Killian Scott B.
Kim Peter
Kircher Susan M.
Kirn Thomas
Klavins Anna
Koeth Laura M.
Kuperus Amanda L.
Lam Christine M.
LaVoie Stephen
Ledesma Carlo
Lee Sang
Leung Beth
Levinson Madison
Lewis James S.
Li Xian-Zhi
Lisboa Luiz
Livesay Hannah
Lozano Sergio
Luna Brian
Lutgring Joseph
Macedo Nubia
Machado Maria Jose
Maddock Kelli
Madon Andrew
Malysa Michelle
Martin Isabella
Mathers Amy J
Matuschek Erika
McCloskey Lynn
McCurdy Sandra
McLeod Sarah
Miller Jennifer
Miller Linda A.

Jones Barb
Jung Sarah
Justo Julie Ann
Kahlmeter Gunnar
Kamau Edwin
Karlsson Asa
Kasapidis Cara
Kersh Ellen N.
Khalid Haziq
Khan Ayesha
Killian Scott B.
Kim Peter
Kircher Susan M.
Kirn Thomas
Klavins Anna
Koeth Laura M.
Kuperus Amanda L.
Lam Christine M.
LaVoie Stephen
Ledesma Carlo
Lee Sang
Leung Beth
Levinson Madison
Lewis James S.
Li Xian-Zhi
Lisboa Luiz
Livesay Hannah
Lozano Sergio
Luna Brian
Lutgring Joseph
Macedo Nubia
Machado Maria Jose
Maddock Kelli
Madon Andrew
Malysa Michelle
Martin Isabella
Mathers Amy J
Matuschek Erika
McCloskey Lynn
McCurdy Sandra
McLeod Sarah
Miller Jennifer
Miller Linda A.

Khan Ayesha
Killian Scott B.
Kircher Susan M.
Kirn Thomas
Klavins Anna
Koeth Laura M.
Kuperus Amanda L.
Lam Christine M.
LaVoie Stephen
Ledesma Carlo
Lee Sang
Leung Beth
Levinson Madison
Lewis James S.
Li Xian-Zhi
Lisboa Luiz
Livesay Hannah
Lozano Sergio
Luna Brian
Lutgring Joseph
Macedo Nubia
Machado Maria Jose
Maddock Kelli
Madon Andrew
Malysa Michelle
Martin Isabella
Mathers Amy J
Matuschek Erika
McCurdy Sandra
McLeod Sarah
Miller Jennifer
Miller Linda A.
Miller William
Minor Sarah
Mitchell Stephanie L.
Moeck Greg
Moore Nicholas M.
Morales Yesenia
Morrissey Ian
Motyl Mary R.
Moussa Samir
Mroz Kaitlyn
Muhia Josephat

Miller William
Min Sharon
Minor Sarah
Mitchell Stephanie L.
Moeck Greg
Moore Nicholas M.
Morales Yesenia
Morrissey Ian
Motyl Mary R.
Moussa Samir
Mroz Kaitlyn
Myers Michelle
Naccache Samia N.
Narayanan Navaneeth
O'Rourke Susan
Ohkusu Kiyofumi
Omori Elena
Onishi Motoyasu
Ordonez Smith de Danies Margaret
Otterson Linda G.
Palavecino Elizabeth
Patel Dimple
Patel Jean B.
Patterson Logan D.
Pham Cau Dinh
Pierce Virginia M.
Pillar Chris
Pischel Kelsey
Quinn Brigit
Rajeev Lara
Ramos Karl Anthony
Razaki Hamid
Re David
Redell Mark A
Rice Felicia
Richter Sandra S.
Robinson Stephanie F.
Rossi Flavia
Rotunno Will
Ruscoe Diane
Russo Carmella
Sabour Sarah
Sanchez Belkys
Miller William
Min Sharon
Minor Sarah
Mitchell Stephanie L.
Moeck Greg
Moore Nicholas M.
Morales Yesenia
Morrissey Ian
Motyl Mary R.
Moussa Samir
Mroz Kaitlyn
Myers Michelle
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Satlin Michael
Scangarella-Oman Nicole
Scheetz Marc H.
Schmerer Matthew
Schneider Cynthia
Schuermeyer Linda
Schuetz Audrey N.
Shannon Samantha
Sharp Susan
Shier Kileen
Shurland Simone M
Simner Patricia J.
Simons Corrie
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Snippes Vagnone Paula M.
Staats Dylan
Stone Gregory G.
Takemura Miki
Tamma Pranita D.
Tekle Tsigereda
Tenllado Jolyn
Thomson Susan
Trauner Andrej
Trebosc Vincent
Turng Ben
Uprety Priyanka
van Nuenen Marc
Van Tam T.
Weingarten Rebecca
Weinstein Melvin P.
Wikler Matthew A.
Winkler Marisa
Wootton Mandy
Xia Dongxiang
Yamano Yoshinori
Yamashiro Hidenori
Yanagihara Katsunori
Yarbrough Melanie
Yee Rebecca
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